



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

005432

JUN 26 1986

OFFICE OF  
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of: 1) Mouse oncogenicity feeding study (with statistical analysis memo from Fisher, 5/13/85) 2) 3-generation rat reproductive study, and 3) 2-generation reproductive study for Dinoseb; Caswell # 392DD; Accession # 259494-259498; EPA # 54299-Q (1)/onco ; Accession # 259499-259506; EPA # 54299-Q (2)/repro

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*6/5/86*

THRU: Laurence D. Chirlik, D.A.B.T.  
Section Head, Section V  
Toxicology Branch/HED (TS-763C)  
and  
Theodore Farber, Ph.D.  
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*6/26/86*

ACTION: Review : 1) Mouse oncogenicity feeding Study 2) 3-generation rat reproductive study, and 3) 2-generation rat reproductive study for Dinoseb

RECOMMENDATIONS:

1. Mouse oncogenicity study

Although reviewed primarily as an oncogenicity study, since the mouse is not considered by the EPA as an acceptable species for chronic toxicity testing, the study failed to establish a NOEL for some toxic or potentially toxic manifestations including: 1) an increase in the rate of development of lenticular opacities in both sexes which was noted by 78 weeks in the mid-and high-dose levels but not investigated in the low dose and 2) adverse effects on the reproductive organs of both sexes including the uterus and testes are suggested by the treatment-related lesions observed in this study. In the uterus there is a consistent increase in the number of lesions observed in the treated females as compared to the controls in all dose groups for cystic endometrial hyperplasia. A similar

*12/2/86*  
*1/6/87*

situation is observed in the test with a report of atrophy/hypospermatogenesis/degeneration and dystrophic calcification in all levels of the dosed males which appears compound-related.

The study reported a statistically significant ( $p < 0.05$ ), treatment-, but not dose-related increase in liver adenomas and adenomas plus carcinomas in treated female mice when the controls were compared against treated mice. Also reported was a statistically significant ( $p < 0.05$ ), treatment-related increase for combined data for these neoplasms (all lesions in both sexes) when compared against the combined control male and female incidences (all lesions). The treated males did not have any statistically significant differences. The study report also noted that in both sexes combined, the incidence for hepatic adenoma in treated mice approached statistical significance ( $p < 0.1$ ). An additional statistical analysis performed by the Toxicology Branch (attached Fisher memo of 5/13/86), and including historical control data, supported the study author's findings. Since the tumors were noted only in the liver and were benign, the biological significance of the increased incidence is highly questionable. Other points which argue against oncogenicity are: 1) the lack of a dose response effect, 2) statistical significance in only one sex and 3) no decrease in the latency period for the development of tumors.

In terms of methodology, a number of tissues/organs were not examined including the trachea, salivary glands, skin, esophagus, colon (cecum was taken), rectum, spinal cord, sternum (femur with bone marrow was taken after 12/21/78), musculature, gall bladder, and aorta. However, these are not considered critical to the determination of histopathological changes which might occur from compound treatment. An important deficiency is the lack of stability data on the stock dinoseb from which the animals were dosed in the dietary feed.

This study is not considered acceptable for chronic toxicity testing in the rodent. If stability data are submitted and found to be acceptable, the rating of Core Supplementary (for oncogenicity) may be upgraded to Core Minimum.

## 2. 3-Generation reproductive study

### a. Reproductive findings:

There is a consistent, compound-related decrease in body weight gain at the high dose in both adult males and females in the pre-mating period in all three generations, which continues in the treated males and females during mating, post-mating, etc. A systemic LEL is thus established at 10 mg/kg/day (LDT) based on depressed parental weight gain and the NOEL is 3 mg/kg/day. The parental systemic LEL of 1 mg/kg/day (LDT) determined in the related 2 generation reproductive study is similar to the parental NOEL (3mg/kg/day) for systemic toxicity determined in this study.

The mean fetal weights were affected by dinoseb administration but with a high degree of variability. Decreased weights were observed or suggested in  $F_0 \rightarrow F_{1b}$ ,  $F_1 \rightarrow F_{2a}$ , and  $F_2 \rightarrow F_{3a}$  littering groups with the  $F_0 \rightarrow F_{1b}$  pup weights diminished (combined sexes) at day 21 at all dose levels compared to controls. Since the pup weights at birth were similar, the decreased pup weight gains at day 21 indicate a reproductive effect of dinoseb related to the lactation period. Based on the findings for decreased pup weights, a reproductive LEL of 1 mg/kg/day is determined and a NOEL was not determined.

b. Teratology

Dinoseb may be fetotoxic but the findings are variable. In the  $F_0(F_{1b})$  pups there was an apparent dose-related increase in the overall skeletal defects ("minor" fetal defects) as compared with the control which was statistically significant at the high dose. For the  $F_2(F_{3b})$  pups there was an apparent compound-related increase (not statistically significant) in the total number of "minor" skeletal defects due primarily to an increase (treatment-related) in sternal and rib defects. However, the  $F_{2b}$  pups did not appear to show any dose- or compound-related effects.

A NOEL cannot be established due to the small number of dams utilized (9 to 10), the lack of litter incidence for fetal defects, and the variability of pre-implantation loss in the controls.

c. Behavioral data

No significant post-natal toxicity is ascribed to dinoseb administration at the doses studied in this assay in light of the small number of animals studied per group, the finding of a small weight change in only one group of rats ( $F_{1b}$  males), and the lack of consistent, statistically significant effects.

With regards to the methodology for the study, a major deficiency in the study was the significant variability of the estimated dosages fed to the animals during the study as well as uncertainty regarding the analysis of the content of the fortified diet and the concentration of compound actually present in the diet. In addition, the report indicated the loss of food records for weeks 9 and 14-65 for both males and females which precludes an accurate estimate of the administered dose.

This study is classified Core Supplementary data.

3. 2-Generation Reproductive Study

In light of: 1) the low viability index for pups in the  $F_4 \rightarrow F_{5a}$  controls (which does not allow a useful comparison of the fetal control data to the treated groups), 2) the inconsistency between the weight changes in the present study (significant weight increases) and the previously reviewed study (significant decreases in three of the six littering groups), and 3) the consistent decrease observed in gonadal weights and organ-to-body weight ratios at all dose levels, it is concluded that a NOEL for reproductive toxicity in the pups can not be established. In addition, the study has failed to establish a systemic NOEL for the weight changes observed in the adults (males or females) and the LEL for systemic toxicity is 1 mg/kg/day (LDT).

An important deficiency in the methods is the lack of stability data on the stock dinoseb from which the animals were dosed in the feed.

This study is designated as Core Supplementary data.

DATA EVALUATION RECORD

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STUDY TYPE: Mouse oncogenicity (100 weeks)

CHEMICAL: Dinoseb, 2-sec butyl 4,6-dinitrophenol (DNBF)

TEST MATERIAL: Technical grade dinoseb; brown crystalline solid (batch # MM 2000 25) of 98.0% purity; blended with the basic powdered diet in a Morton '50E' batch mixer or a Gardner double cone blender.

Nominal dose levels: 0, 1, 3, 10 mg/kg/day

Animals used: albino mice of CD-1 strain; 70/dose level per sex with 10 mice per sex for a 6 and 12 month interim sacrifice

STUDY IDENTIFICATION:

a. Title: "Dinoseb: A 100 week oral (dietary) toxicity and carcinogenicity study in the mouse"

b. Laboratory: Hazleton Laboratories Europe Ltd.,  
Otley Road,  
Harrogate, HG3 1PY,  
England

c. Study Number: 1839-50/20

d. Study Date: June 1981

e. Study Director: D. Brown, B.Sc., D.Phil.,  
Associate Director of Toxicology

f. Caswell # 392DD; Accession # 259494-259498; EPA # 54299-Q (1)

CONCLUSIONS:

Although reviewed primarily as an oncogenicity study, since the mouse is usually not considered by the EPA as an acceptable species for chronic toxicity testing, the study failed to establish a NOEL for a number of toxic or potentially toxic manifestations including: 1) an increase in the rate of development of lenticular opacities in both sexes which was noted by 78 weeks in the mid-and high-dose levels but not investigated in the low dose and 2) adverse effects on the reproductive organs of both sexes, including the uterus and testes, are suggested by the treatment-related lesions observed in this study. In the uterus there is a consistent increase in the number of lesions observed in the treated females as compared to the controls in all dose groups for cystic endometrial hyperplasia. A similar situation is observed in the testes with a report of atrophy/hypospERMATogenesis/degeneration and dystrophic calcification in all levels of the dosed males, which appears compound-related.

The study reported a statistically significant ( $p < 0.05$ ), treatment-, but not dose-related increase in liver adenomas and adenomas plus carcinomas in treated female mice when the controls were compared against treated mice. Also reported was a statistically significant ( $p < 0.05$ ), treatment-related increase for combined data for these neoplasms (all lesions in both sexes) when compared against the

combined control male and female incidences (all lesions). The treated males did not have any statistically significant differences. The study report also noted that in both sexes combined, the incidence for hepatic adenoma in treated mice approached statistical significance ( $p < 0.1$ ). An additional statistical analysis performed by the Toxicology Branch, and including historical control data, supported the study author's findings. Since the tumors were noted only in the liver and were benign, the biological significance of the increased incidence is highly questionable. Other points which argue against oncogenicity are: 1) the lack of a dose response effect, 2) statistical significance in only one sex and 3) no decrease in the latency period for the development of tumors.

In terms of methodology, a number of tissues/organs were not examined including the trachea, salivary glands, skin, esophagus, colon(cecum was taken), rectum, spinal cord, sternum (femur with bone marrow was taken after 12/21/78), musculature, gall bladder, and aorta. However, these are not considered critical to the determination of histopathological changes which might occur from compound treatment. An important deficiency is the lack of stability data on the stock dinoseb from which the animals were dosed in the dietary feed.

This study is not considered acceptable for chronic toxicity testing in the rodent. If stability data are submitted and found to be acceptable, the rating of Core Supplementary (for oncogenicity) may be upgraded to Core Minimum.

METHODS:

A copy of the methods section from the study is attached. This study was performed under the then proposed EPA Toxicology Guidelines(1978) for Registering Pesticides in the U.S.(p. 719, Volume II), therefore the procedures will be basically evaluated on that basis. The study will be evaluated primarily as an oncogenicity study and not as a chronic toxicity study as well, since the mouse is not generally regarded by the EPA as an acceptable species for chronic toxicity testing (p. 37375 of 1978 Guidelines) and the rationale for selection of the mouse as the specie of choice was not given. This justification for use of an alternative mammalian species is also requested in the final 1982 EPA Guidelines.

The following comments are presented:

1. A deficiency in the study protocol is a number of tissues/organs not examined by the test facility, which are recommended by either the 1978 or the 1982 EPA Guidelines. However, these are not consider critical to the histopathology evaluation (personal communication from L. Kasza; April, 1986). They include the following:

Tissues/organs Required by: 1978 Guidelines?(p.37376) 1982 Guideline?(p.113-115)

trachea	Yes	Yes
salivary glands	"	"
skin	"	"
esophagus	"	"
jejunum, colon, rectum	---	"
spinal cord	" (at 2 levels)	" (at 3 levels)
musculature	"	"
gall bladder	"	"
aorta	No	Yes

2. A MTD (maximum tolerated dose) appears to have been established with the high dose group (10 mg/kg/day) for both the male and female mice. Although the group mean male body weight increases appeared similar to the controls throughout the study (see Table 4 and Figure 2 in study report) an examination of food conversion (Table 1 of review) and the incidence of lenticular opacities (Table 4 of review) indicate a biological effect without the production of excessive mortality--in fact, group survival for both the males, and possibly the females, appears to be increased in the mid and high dose groups. The females in the medium and high dose groups have a dose-related reduction in the rates of body weight gain:

Dose	0-26wks	0-52wks	0-78wks	0-98wks
Control	12.43g	19.54g	21.56g	21.64g
Low	11.52g	18.02g	23.00g	24.40g
Medium	10.91g	16.46g*	19.69g	17.35g*
High	8.97g*	14.30g**	16.69g*	17.14g*

(\*p<0.05; \*\*p<0.01; Student's t-test using an estimate of the S.D. derived from the analysis of variance)

3. It is unusual to perform hematology and clinical chemistry of any extent, particularly for serial samples, in such a small animal as the mouse. This difficulty is highlighted by the admitted problems encountered and reported by the experimenters on pages 8 and 9 of Volume I, i.e., in the 25 week bleed where insufficient blood was obtained or no blood obtained from the animals.

4. The temperature and humidity ranges for the environmental controls are quite large (12-30 degrees Celsius and 29-96% relative humidity, p. 6. vol. I) but do not appear to have significantly affected the study.

5. The stability and homogeneity of the test substance are of particular importance in a long-term test:

a. It is unclear as to whether the stability of the stock dinoseb itself was determined; this is critical since all the dietary mix was prepared from a single batch of test material shipped from the manufacturer, Dow Chemical Pacific Ltd. (p.11, Volume I). Edgerton and Moseman (J.Agric.Chem.,26(2):425,1975) observed that their DNBP analytical standards significantly degraded (27% loss after 72 hrs) when stored in clear glass bottles. Were the internal standards of DNOC (4,6-dinitro-o-cresol) and DNBP used in the calibration curves and subsequent dietary stability tests (DNOC only) adequately controlled for chemical degradation over the life of the study?

b. The test laboratory experienced some difficulty in both the analysis and homogeneity of the dietary feeding mixture. The analytical method appeared to be satisfactorily resolved but some difficulty in the homogeneity of the diet mixture was observed as evidenced by a disparity in duplicate samples as indicated below:

<u>Sample</u>	<u>DNBP found (ug/g)</u> <u>(% of dsb intended)</u>	<u>Intended concentration</u>
(Table 18/week 13)		
low dose females		
1	9.2 (153)	6.0
2	7.9 (132)	6.0
(Table 19/week 26)		
medium dose males		
1	18.9 (99)	19.1
2	30.8 (161)	19.1
high dose females		
1	46.6 (74)	62.6
2	70.2 (112)	62.6
(Table 20/week 39)		
low males		
1	4.9 (58)	8.4
2	7.6 (90)	8.4
medium dose males		
1	29.9 (116)	25.8
2	19.6 (76)	25.8
(Table 21/week 54)		
low dose males		
1	5.2 (68)	7.7
2	3.9 (51)	7.7
high dose females		
1	90.3 (108)	83.4
2	71.1 (85)	83.4
(Table 22/week 67)		
high dose females		
1	159.9 (160)	100.0
2	124.0 (124)	100.0

(For weeks 78 and 91 of sampling: the dietary concentrations were usually equivalent)

The variability in the test concentrations is significant with some values as high as 161% or as low as 51% of the intended dose. However, it should be noted (p. 28) that overall the nominal concentrations in the diet were within  $\pm 10\%$  of the actual measured dietary concentration.

6. Summary tables for organ weights (absolute or relative) were not provided.

7. A summary table for all non-neoplastic histopathology was not provided.

#### RESULTS:

##### 1. Morbidity and Mortality; Clinical Signs

As mentioned previously, the longevity of the males in the medium and high dose groups, and possibly of the females, appeared to be increased.

Some of the mice developed opacities of the eye, this will be discussed more fully below. Also, a number of mice (both sexes) developed subcutaneous tissue masses (see Table 11 of report). These masses were reported as superficial,

frequently hard, compact and quite mobile. In many instances the masses regressed within a few weeks. The incidence of masses was not treatment- or dose-related and the authors attributed the masses to small abscesses resulting from bites. The reviewer is inclined to agree in light of the aggressive nature of mice, particularly under the conditions of group housing.

Clinical signs were summarized by the investigators for each animal in the gross and microscopic pathology tables of the report. No unusual signs were reported except for an increased incidence of yellow staining of the fur which appeared to be dose-related, and possibly sex-related (100% staining of the fur in males at week 90 as opposed to only 50% in the females; see Table 3 of study). The staining was reported as mainly involving the head, neck and fore limbs and not associated with any apparent increase in morbidity. This effect is due to the coloring properties of dinoseb.

## 2. Body Weight Changes

This has been addressed under the Methods section (p.3 of review, discussion of MTD). See below (Section 3.) for a discussion of body weights relative to food consumption (Table 1).

## 3. Food Consumption/Food Conversion

Food consumption based on the amount consumed (g) per day (group mean) was reported as similar for all the experimental groups during the study (see Table 4, pages 52-84 of report) although the data were not tabulated in a way which allowed for a convenient comparison of the results, i.e., averages with ranges and statistical analysis. The reviewer calculated the food conversion, a measure of the efficiency of conversion of ingested food into body mass (food consumption divided by the body weight gained in a given interval). The data are presented below in Table 1 of this review:

Table 1

Food conversion: total food consumed in g -- b. wt. gained (g) in interval(weeks=w)

	<u>W0-W20</u>	<u>W0-W40</u>	<u>W0-W60</u>	<u>W0-W80</u>	<u>W0-W99</u>
1 M (control)	11.63	14.76	19.20	25.87	36.84
1 F	11.20	11.41	14.32	18.75	22.90
2 M (1 mg/kg/d)	11.67	16.88	20.60	25.56	35.22
2 F	12.03	13.84	15.61	18.37	21.53
3 M (3 mg/kg/d)	9.95	16.26	22.22	27.76	41.48
3 F	11.80	13.58	17.45	21.49	29.00
4 M (10 mg/kg/d)	10.75	15.93	20.38	27.20	42.03
4 F	14.32	15.89	20.02	28.07	35.02

M=male, F=female.

005432

Several observations are possible. First, it can be noted that food conversion values increase as the length of the study progresses--an indication that the animals' body is less efficient at converting the ingested food into body mass, muscle, fat, etc., as it ages. This is to be expected. There also appears to be a treatment-related effect on food conversion (decrease in efficiency) at the medium and high dose levels over and above that observed in the controls but the low dose group values (both sexes) appear quite similar to the controls. A dose-related effect is also noted in that the high dose appears to produce a change in food conversion earlier than the medium dose [approximately by Week 20 (females) as compared to Week 40 (males and females), respectively]. This effect is consistently greater in the females of both of these dose groups, although more pronounced in the 10 mg/kg/day dose group. Thus, on the basis of effects on the efficiency of food conversion, a NOEL of 1 mg/kg/day is suggested. These effects were not analyzed by the reviewer for statistical significance.

#### 4. Hematology; Clinical Chemistry; Urinalysis

Overall, no unusual changes were noted in the hematology parameters. Clinical chemistries indicated some increases in alkaline phosphatase (Iu/l) [none statistically different] in the treated males but apparently not in the females (see below):

	13wks	25wks	52wks	78wks	98wks
0 mg/kg					
males	96(20) <sup>a</sup>	108(37)	93(39)	128(32)	165(120)
females	180(11)	111(54)	103(29)	164(103)	124(61)
10 mg/kg					
males/	119(38)	152(58)	132(56)	189(143)	451(602)
females	137(52)	132(95)	96(18)	136(52)	108(53)

(<sup>a</sup>=S.D.)

It is unclear whether these are biologically relevant since, as noted by the investigators, a few high plasma enzyme activities resulted in the higher readings. For example, in the high dose group, one male animal at week 78 had a reading of 528 (# 233) and three males at 98 weeks had values of 691, 870 and 1966 (#'s 240, 243, and 251). Removal of these four values would have resulted in a mean of 151 and 141, respectively.

Examination of the urinalysis data did not reveal any unusual findings for either the male or female mice.

005432

Table 2  
Organ Weights: Absolute=A(g), Relative=R(%)  
(Terminal Sacrifice)

		Brain	Liver	Heart	Gonads		Adrenals		Kidneys		Lung
					Left	Right	Left	Right	Left	Right	
1M	A	.50 (.033) <sup>a</sup>	2.53 (1.373)	.26 (.055)	.105 (.024)	.110 (.03)	.004 (.002)	.005 (.001)	.33 (.07)	.34 (.072)	.26 (.026)
	R	1.195 (.158)	6.247 (4.038)	.618 (.106)	.253 (.054)	.262 (.066)	.011 (.006)	.011 (.003)	.797 (.151)	.804 (.158)	.62 (.077)
1F	A	.50 (.042)	1.78 (1.343)	.18 (.029)	.112 (.227)	.136 (.188)	.007 (.002)	.006 (.002)	.23 (.053)	.23 (.044)	.32 (.279)
	R	1.282 (.301)	4.432 (.916)	.448 (.088)	.287 (.627)	.331 (.482)	.017 (.007)	.016 (.006)	.593 (.223)	.584 (.151)	.87 (.986)
2M	A	.48 (.044)	2.24 (.758)	.23 (.036)	.101 (.027)	.108 (.027)	.007 (.014)	.004 (.001)	.33 (.037)	.34 (.056)	.28 (.07)
	R	1.121 (.221)	5.09 (1.514)	.543 (.149)	.232 (.064)	.249 (.073)	.015 (.023)	.010 (.005)	.768 (.145)	.775 (.135)	.66 (.248)
2F	A	.50 (.043)	2.13 (1.268)	.19 (.035)	.377 (.453)	.201 (.256)	.005 (.002)	.005 (.002)	.22 (.037)	.24 (.036)	.25 (.053)
	R	1.208 (.265)	5.113 (3.544)	.451 (.123)	.899 (1.116)	.477 (.673)	.012 (.006)	.013 (.006)	.535 (.131)	.561 (.123)	.61 (.184)
3M	A	.49 (.023)	2.76 (1.809)	.27 (.093)	.107 (.024)	.101 (.031)	.005 (.002)	.005 (.003)	.38 (.201)	.34 (.063)	.32 (.138)
	R	1.246 (.141)	6.871 (4.533)	.71 (.337)	.272 (.06)	.255 (.072)	.013 (.006)	.013 (.007)	.952 (.489)	.842 (.098)	.84 (.468)
3F	A	.51 (.039)	1.74 (.635)	.18 (.039)	.511 (2.03)	.107 (.185)	.005 (.002)	.005 (.002)	.23 (.046)	.23 (.045)	.28 (.152)
	R	1.498 (.311)	4.877 (1.381)	.51 (.135)	1.4 (5.637)	.296 (.492)	.016 (.006)	.014 (.006)	.648 (.131)	.655 (.119)	.81 (.403)
4M	A	.48 (.044)	3.56 (2.219)	.23 (.027)	.101 (.022)	.097 (.02)	.004 (.002)	.004 (.002)	.34 (.088)	.36 (.082)	.34 (.208)
	R	1.22 (.174)	9.167 (5.883)	.583 (.07)	.259 (.063)	.250 (.063)	.009 (.004)	.011 (.005)	.864 (.225)	.910 (.200)	.88 (.618)
4F	A	.50 (.047)	1.89 (.686)	.17 (.041)	.355 (1.215)	.116 (.281)	.006 (.003)	.005 (.003)	.21 (.048)	.22 (.05)	.26 (.152)
	R	1.503 (.341)	5.489 (1.88)	.511 (.143)	1.1 (3.919)	.319 (.703)	.017 (.011)	.016 (.01)	.615 (.135)	.651 (.14)	.77 (.453)

<sup>a</sup> Value (S.D.); M= male, F= female; 1=0 mg/kg/day, 2=1 mg/kg/day, 3=3 mg/kg/day, 4=10 mg/kg/day (nominal concentrations)

005432

Table 3  
Relative Organ Weights (% b.wt.)  
6 Month Sacrifices

	Brain	Liver	Heart	Gonads		Adrenals		Kidneys		Lung
				Left	Right	Left	Right	Left	Right	
1 M	1.26 (.16) <sup>a</sup>	4.84 (.655)	.60 (.105)	.336 (.047)	.341 (.044)	.014 (.005)	.014 (.006)	.88 (.138)	.87 (.162)	.58 (.093)
1 F	1.63 (.171)	5.31 (.611)	.47 (.074)	.043 (.02)	.044 (.025)	.020 (.009)	.019 (.008)	.59 (.117)	.61 (.093)	.63 (.081)
2 M	1.27 (.156)	4.86 (.734)	.69 (.098)	.347 (.062)	.339 (.058)	.011 (.004)	.013 (.003)	.85 (.12)	.85 (.009)	.58 (.08)
2 F	1.57 (.175)	5.09 (.761)	.61 (.144)	.039 (.011)	.040 (.015)	.022 (.009)	.016 (.006)	.63 (.093)	.61 (.066)	.66 (.081)
3 M	1.15 (.135)	4.82 (.763)	.58 (.089)	.321 (.036)	.325 (.03)	.013 (.006)	.013 (.008)	.77 (.104)	.80 (.131)	.58 (.114)
3 F	1.53 (.237)	4.83 (1.172)	.54 (.074)	.042 (.018)	.04 (.009)	.021 (.008)	.02 (.006)	.61 (.066)	.62 (.072)	.64 (.086)
4 M	1.21 (.15)	4.48 (1.242)	.58 (.132)	.332 (.045)	.319 (.063)	.012 (.005)	.013 (.005)	.85 (.102)	.85 (.1)	.57 (.062)
4 F	1.72 (.189)	5.23 (.891)	.57 (.139)	.045 (.012)	.040 (.012)	.020 (.007)	.021 (.007)	.64 (.071)	.64 (.072)	.66 (.054)

12 Month Sacrifices

	Brain	Liver	Heart	Gonads		Adrenals		Kidneys		Lung
				Left	Right	Left	Right	Left	Right	
1 M	1.026 (.131)	4.869 (.591)	.486 (.09)	.277 (.073)	.265 (.061)	.018 (.012)	.016 (.008)	.631 (.094)	.675 (.096)	.54 (.072)
1 F	1.322 (.244)	4.624 (.592)	.436 (.059)	.056 (.066)	.050 (.045)	.020 (.008)	.017 (.005)	.555 (.075)	.576 (.069)	.57 (.102)
2 M	1.146 (.175)	5.519 (1.23)	.541 (.109)	.275 (.079)	.293 (.046)	.011 (.004)	.011 (.007)	.751 (.161)	.762 (.154)	.55 (.159)
2 F	1.205 (.261)	5.604 (3.866)	.452 (.147)	.053 (.076)	.025 (.008)	.018 (.007)	.020 (.01)	.565 (.281)	.577 (.257)	.63 (.393)
3 M	1.04 (.07)	5.358 (.591)	.507 (.117)	.245 (.031)	.249 (.037)	.014 (.006)	.014 (.005)	.783 (.109)	.797 (.081)	.61 (.303)
3 F	1.408 (.227)	5.122 (.708)	.430 (.11)	.053 (.056)	.052 (.07)	.017 (.007)	.018 (.007)	.551 (.081)	.559 (.088)	.56 (.105)
4 M	1.115 (.10)	4.577 (.413)	.475 (.029)	.308 (.08)	.298 (.071)	.011 (.004)	.012 (.005)	.745 (.076)	.762 (.091)	.58 (.054)
4 F	1.385 (.164)	5.398 (1.052)	.435 (.061)	.031 (.033)	.028 (.026)	.020 (.009)	.019 (.011)	.564 (.084)	.590 (.108)	.66 (.164)

a Value (S.D.); M=male, F=female; 1=0, 2=1, 3=3, 4=10mg/kg/day, resp.

## 5. Organ Weights

Summaries of the absolute and relative organ weights (terminal sacrifice) and relative organ weights (6 and 12 month sacrifices) for tissues of concern are presented above in Tables 2 and 3.

Relative organ weights (Table 3) at 6 month sacrifices were not different for either treated males or females from control values. There was a weak suggestion of a possible dose-related increase (mid, high) in brain relative weights (Table 3) in females at 12 months which most likely relates to the diminished body weights of the animals since absolute brain mean weights were not different in the treated as compared to the controls. This effect was still observed in the terminal sacrifices (Table 2). Relative kidney weights of the males at 12 months also appear to be similarly affected at all three dose levels in both the right and left sides and this effect is still present at terminal sacrifice (both sides). Again, this probably results to animal weight reduction since absolute mean kidney weights in the treated animals were similar to the controls. While kidney weights do not appear to be increased at 12 months in the females, there is a suggestion of an increase, based on their relative weights, at the two higher doses (both sides) at terminal sacrifice (Table 2). Again, this probably relates to the decreased total body weight since the absolute kidney weights at both the mid- and high-dose females were the same as controls.

There appears to be dose-related effects in both sexes for the liver (absolute and relative weights) at terminal sacrifice in the mid- and high dose in males and the low and high doses in the females. The investigators have suggested that this is related to a small number of extra heavy livers (they only state the high dose as being greater than the controls), however, in the case of the high-dose males, there were six individual relative weights(g) ranging from 9.866-24.206, and two approximately 7.0, for a total of 8/14 livers (14 males survived to terminal sacrifice with ratios greater than the mean control). For the high-dose females there was only one apparent aberrant ratio (13.597). Averaging of the remaining 26 values gave a relative organ weight of 5.178 which is still greater than the control value (4.432). Therefore, the effects noted in the liver, even though of a somewhat erratic nature, do appear to be treatment and dose-related phenomenon. Examination of the absolute or relative liver weights of females in the high-dose groups with liver adenomas does not suggest any correlation between liver weight changes and liver tumors.

## 6. Lenticular Opacities

Dinitrophenols have been associated with lenticular opacities (i.e., cataracts) in humans since their use in the 1930's as weight reducers and are cataractogenic in ducklings and young rabbits (W.J. Hayes, Pesticides Studied in Man, 1982). Dinitrophenol has been reported in one study to produce cataracts in ducklings (Spencer et al., J. Ind. Hyg. Toxicol, 1948). Therefore, it is not surprising to find that the compound promotes lenticular opacities in mice (see Table 4 below).

By 78 weeks of exposure a treatment-related increase in the development of l.o.'s was observed in the mid- and high-dose groups of both sexes. As a result of the findings in week 78, animals from the high dose group were also

Table 4  
Incidence of lenticular opacities  
Nominal Dose (mg/kg/day)

Observation Period(wks)	Group 1		Group 2 [Incidence (%)]		Group 3		Group 4	
	Males	Females	Males	Females	Males	Females	Males	Females
18	2/70(2.9)	1/70(1.4)	---	---	---	---	3/70(4.3)	1/69(1.4)
28	3/57(3.5)	1/60(1.7)	---	---	---	---	3/60*(5)	1/59(1.7)
53	2/49(4.1)	3/55(5.5)	---	---	---	---	0/58(0)	1/56(1.8)
78/79	1/31(3.2)	5/37(13.5)	---	---	13/37 (35.1)	19/42 (45.2)	4/30 (13.3)	20/37(54.1)
83	---	---	---	---	---	---	13/24 (54.2)	16/25(64)
99	11/13 (84.6)	24/24 (100)	18/20 (90)	24/25 (96)	18/18 (100)	24/25 (96)	14/15 (93.3)	28/28(100)

\* total # animals miscounted in report (Table 9, p.93)

examined at 83 weeks by an independent veterinary ophthalmologist for the purpose of establishing the sponsor's diagnosis (see Table 4). This diagnosis confirmed the original diagnosis by the study pathologist. Males and females in the high dose groups had increased incidences of l.o.s as compared to the mid-dose groups at weeks 78/79. All groups including the controls had an almost 100% l.o. rate by the end of the study, however comparison of the severity of the l.o.'s for the various groups indicated an increase in the severity of the lenticular lesions (in terms of density and extent) at the high dose in both sexes:

	A (0 mg/kg)B		A (10 mg/kg)B	
Males	1.7	2.5	2.5**	3.4**
Females	1.9	3.1	3.0**	3.6*

A= density of opacity  
B= area of opacity

(Group Mean Scores)

\*p<0.05; \*\*p<0.01

The effect of Dinoseb is quite severe and one wonders if the animals were not indeed completely blind as evidenced by the comment in the study report (p. 38) that, "At the termination of the study the majority of these mice had lenses which were almost completely opaque over most of their surface area". No examination of low dose(1 mg/kg) mice was performed at the 78 week observation period. In light of the significant response at the medium dose, such an effort should have been undertaken to determine if the low dose had resulted in an increased rate of lenticular opacities at this period. This would have allowed a possible determination of a NOEL for this effect.

Table 5 (taken in part from Table 4 of attached memorandum)  
Female liver tumor data

ADENOMAS						Animals <sup>†</sup>
Dose(mg/kg/d)	weeks: 27*	54/55	56-99	100/101	Total	on test
0		-----	-----	-----	-----	70(68:1x)
1		-----	-----	3	3	70(69:1a)
3		-----	2	5	7	70
10		-----	-----	5	5	70(68:1a, 1x)
CARCINOMAS						
Dose(mg/kg/d)	weeks: 27*	54/55	56-99	100/101	Total	
0		-----	-----	-----	-----	70(68:1x)
1		-----	-----	1	1	70(69:1a)
3		-----	-----	-----	-----	70
10		-----	-----	-----	-----	70(68:1a, 1x)
ADENOMAS & CARCINOMAS						
Dose(mg/kg/d)	weeks: 27*	54/55	56-99	100/101	Total	
0		-----	-----	-----	-----	70(68:1x)
1		-----	-----	4	4	70(69:1a)
3		-----	2	5	7	70
10		-----	-----	5	5	70(68:1a, 1x)

\* individual animal histopathology not reported; † number does not reflect liver tissues which were lost (due to missing animals= x) or autolyzed (a); numbers in parentheses are corrected values

b. Non-neoplastic Lesions: Table 6

Since no summary of non-neoplastic lesions was provided in the study report, lesions/observations of possible relevance were summarized by this reviewer for each treatment group by sexes (see Table 6)--all observations noted during the entire study, including interim deaths and sacrifices, have been presented.

Amyloidosis (a deposition of amyloid in the body, a waxy translucent substance consisting of protein in combination with polysaccharides) was prevalent in a number of organs and tissues particularly the adrenal gland, ileum, kidneys, liver, and thyroid. There appeared to be a dose-related effect in the males but not the females, reflected in the total observations for all tissues and organs (i.e., control= 42, 1 mg/kg= 79, 3 mg/kg=113, and 10 mg/kg=66) which appeared to diminish at the highest dose (see Table 6). Whether this is a true effect would require an evaluation of the incidence (# of animals per dose with the finding).

In the liver there is a suggestion of increased necrosis at the mid- and high dose levels (both sexes) as compared to controls (see Table 6). The effect in liver is relevant to the issue of the proper establishment of an MTD and the apparent treatment-related increase in liver tumors in the female mice (see p. 17 of this review for further discussion) since liver necrosis suggests that the dose administered may have altered the normal physiological conditions of the hepatic cells.

Results from the kidneys and lungs are not suggestive of a toxic response from dinoseb administration.

A consistent, treatment- but not dose-related effect in both sexes in the thymus is indicated from the data-- primarily in terms of involution or atrophy of the tissue -- at all dose levels. The changes appear to be somewhat more predominant in the females than the males. However since thymus involution is normal, the meaning of this observation is uncertain--although it could relate to an effect of Dinoseb on the immune system.

Dinoseb has been reported to produce reproductive effects in mice including resorptions, reduced size of the young, fetotoxicity, and teratogenicity ( W.J. Hayes, Pesticides Studied in Man, 1982, p.472). Adverse effects on the reproductive organs of both sexes including the uterus and testes are suggested by the treatment-related lesions observed in this study. In the uterus there is a consistent, similar increase in the incidence of cystic endometrial hyperplasia observed in all dose groups of treated females as compared to the controls (Table 6). An increased incidence of lesions is also observed in the testes with a report of atrophy/hypospermatogenesis/degeneration in all levels of dosed males which appears compound-related.

Table 6  
Non-Neoplastic Lesions\*  
(Total of 70 animals/sex/group)

Amyloidosis (organs/tissues)	0 mg/kg		1mg/kg		3mg/kg		10 mg/kg	
	Male	Female	Male	Female	Male	Female	Male	Female
adrenal gland	6	16	13	7	14	15	10	6
cecum	1	1	—	—	1	—	2	1
duodenum	4	10	6	3	8	10	3	4
eye/optic nerve	—	1	—	—	—	—	—	—
heart	2	6	6	—	10	7	5	—
ileum	3	13	10	9	13	19	7	10
kidneys	6	16	13	11	17	19	13	—
liver	5	12	7	3	12	10	7	3
lungs(interstitial)	1	1	—	—	—	—	1	1
lymph nodes(cervical)	—	—	—	—	—	1	2	—
lymph nodes(mesenteric)	—	—	2	—	—	—	1	—
ovaries	—	9	—	9	—	14	—	7
pancreas	1	1	2	—	7	3	1	—
prostate	—	—	—	—	1	—	—	—
spleen	4	5	4	2	7	6	2	1
stomach	—	2	—	—	—	4	1	3
testes	2	—	3	—	9	—	1	—
thymus	—	—	—	—	—	1	—	—
thyroid(interstitial, peri-follicular)	5	11	10	8	13	17	8	6
uterus	—	3	—	1	—	—	—	1
salivary	—	1	2	—	1	3	2	1
submaxillary	—	—	1	—	—	—	—	—
jejunum	2	1	—	1	—	—	—	—
Total(each sex)	42	109	79	54	113	129	66	44
Combined (both sexes)	151		133		242		110	
<u>Liver</u>								
hepatocellular necrosis								
Total (each sex)	6	2	6	3	8	6	12	6
Combined (both sexes)	8		9		14		18	
<u>Thymus</u>								
involution (atrophy)	10	12	18	25	16	31	18	23
medullary pigmentation	—	1	—	1	—	—	—	1
lymphoid hyperplasia/inflam.	—	—	—	2	—	6	1	6
thrombus	—	—	—	—	—	1	—	—
angiectasis	—	—	—	—	—	1	—	—
congestion	—	—	—	2	—	—	2	1

Table 6 (continued)

Uterus	0 mg/kg		1mg/kg		3mg/kg		10 mg/kg	
	Male	Female	Male	Female	Male	Female	Male	Female
cystic endometrial hyperplasia		20		27		30		29
congestion		1		2		1		—
acute periarteritis		—		1		—		—
endometrial cyst ; focal endo-		4		2		5		2
metrial cyst								
perivasculitis/periarteritis		1		3		—		1
adenomyosis		—		4		—		2
focal angiectasis/thrombus/pigmen-								
tation		—		1		1		1
glandular squamous metaplasia		—		—		2		1
luminal distension		1		—		—		3
stromal hyperplasia		—		—		—		1
endometrial stromal polyp		—		—		—		1
dystrophic calcification		—		—		—		1
degeneration/necrosis		—		—		—		1
endometritis/myometritis		2		—		—		1
<u>Ovaries</u>								
cystic ovarian bursa		10		10		1		2
ovarian cyst		16		22		18		18
fallopian tube ectasia		—		1		—		—
periovarian steatitis		—		1		—		—
hematocyst/focal hemorrhage		1		7		2		4
abscess		—		—		1		—
granulosa cell hyperplasia		—		1		1		—
atrophy		3		2		3		4
focal pigmentation		1		2		—		2
congestion		—		2		—		—
fibrinoid degeneration		—		—		1		—
focal arteriolar degeneration		—		—		—		1
oophoritis		—		—		—		1
thrombus		1		1		—		—
mineralization/calcification		1		1		—		—
cholesterol cleft formation		1		1		—		—
luteinization		—		1		—		—
panarteritis		—		1		1		—
<u>Testes</u>								
unilateral fibrosis		—		1		—		—
" " mineralization		—		1		—		—
atrophy/hypospermatogenesis/ degeneration		8		18		28		15

Table 6 (continued)

Testes (continued)

	0 mg/kg		1mg/kg		3mg/kg		10 mg/kg	
	Male	Female	Male	Female	Male	Female	Male	Female
interstitial cell hyperplasia	8		8		1		--	
focal mineralization	4		6		--		5	
suppurative orchitis	1		--		--		--	
lymphocytic perivascularitis/ periarteritis	--		2		2		1	
dystrophic calcification	--		4		8		4	
hemorrhage	--		1		--		--	
spermatocoele	--		--		1		--	

\* Histomorphological observations at 52 weeks, during the study, and/or the conclusion of the study

DISCUSSION:

The study was evaluated primarily as an oncogenicity study and not as a chronic toxicity study as well, since the mouse is not usually considered by the EPA as an appropriate species for chronic testing (p. 37375 of 1978 Guidelines) and the rationale for selection of the mouse as the species of choice for chronic toxicity evaluation was not given. This justification for use of an alternative mammalian species is also requested in the final 1982 EPA Guidelines.

In terms of methodology, a number of tissues/organs were not examined including the trachea, salivary glands, skin, esophagus, colon(cecum was taken), rectum, spinal cord, sternum (femur with bone marrow was taken after 12/21/78), musculature, gall bladder, and aorta. However, these are not considered critical to the determination of histopathological changes which might occur from compound treatment. An important deficiency is the lack of stability data on the stock dinoseb from which the animals were dosed in the dietary feed.

A MTD (maximum tolerated dose) appears to have been established with the high dose group (10 mg/kg/day) for both the male and female mice based on reduced food conversion efficiency, the presence of lenticular opacities in males and females and reduced body weight gains in the females. Although the rates of body weight increase for treated mice were essentially similar to the controls throughout the study (see Table 4 and Figure 2 in the study report), an examination of food conversion (Table 1 of review) and the incidence of lenticular opacities (Table 4 of review) indicates a biological effect without the production of excessive mortality--in fact group survival for both the males, and possibly the females, appears to be increased in the mid- and high-dose groups. The females in the medium and high dose groups have a dose-related reduction in the rates of body weight gain. There is an indication of hepatic necrosis at the mid- and high-doses (see Table 6) in both sexes, suggesting that dinoseb may have produced toxic conditions in the liver with alteration of physiological conditions such that there could have been a qualitative effect on the induction of the observed liver tumors (see OSTP 1984: Fed.Reg. Vol.49 No. 100, 21635). No excess necrosis is suggested in the low dose group where the tumors appear elevated also.

Although reviewed primarily as an oncogenicity study, since the mouse is usually not considered by the EPA as an appropriate species for chronic toxicity testing, the study failed to establish a NOEL for a number of toxic or potentially

toxic effects. While a NOEL related to food consumption data [food conversion (see page 5)--a measure of the conversion of ingested food into body mass] appears established, the study did not establish a NOEL for the following: 1) an increase in the rate of development of lenticular opacities in both sexes which was noted by 78 weeks in the mid- and high-dose levels but not evaluated in the low dose and 2) adverse effects on the reproductive organs of both sexes including the uterus and testes which are suggested by the treatment-related lesions observed in this study. In the uterus there is a consistent increase in the number of lesions observed in the treated females as compared to the controls in all dose groups for cystic endometrial hyperplasia. A similar situation is observed in the testes with a report of atrophy/hypospermatogenesis/degeneration and dystrophic calcification in the dosed males which appears compound-related.

A statistically significant ( $p < 0.05$ ), treatment, but not dose-related, increase in liver adenomas and adenomas plus carcinomas was reported in the study in treated female mice when the controls were compared against treated mice. Also reported was a statistically significant ( $p < 0.05$ ), treatment-related increase for combined data for these neoplasms (all lesions in both sexes) when compared against the combined control male and female incidences (all lesions). The treated males did not have any statistically significant differences. When comparing the incidences of adenoma for control versus total treated mice, there was a slightly higher treated incidence (control= 15.7%, treated= 23.3%). The study report also noted that in both sexes combined, the incidence for adenoma in treated mice approached statistical significance ( $p < 0.1$ ). There did not appear to be any treatment-related decrease in the latency period for development of the liver adenomas or carcinomas. An additional statistical analysis performed to confirm the findings of the study report supported the original analysis as well as indicating that the addition of historical control data did not alter the findings.

Although there were statistically significant increases reported in the female mice for liver adenomas and adenomas plus carcinomas reported, it is questionable whether this constitutes a true oncogenic response which is of biological significance for the following reasons:

- ° the tumors are not induced in a dose-dependent manner, i.e., the response is not a function of the concentration at the presumed target organ--a basic assumption of toxicological experimentation
- ° the tumors are found only in one sex (females)
- ° the tumors are basically benign, and not life threatening
- ° there is no decrease in the latency period for the development of this tumor
- ° the small elevation of hepatic necrosis at the mid- and high-dose groups suggests that severe tissue/organ injury may have been produced. Dosing at a concentration which may produce severe toxic insult could result in an aberrant effect on liver DNA such as increased methylation, altered metabolism and pharmacodynamic parameters (O. Paynter, S.E.P. for Oncogenicity, June, 1985) which could promote an oncogenic response not likely to be seen at lower, less toxic concentrations

° in the females there is a slight increase in the survival time at all dose levels over the control animals (control= 30% vs low dose= 34%, mid-dose= 33%, and high-dose= 39%) which could allow more time for the expression of latent tumors. This is suggested by examination of the time tumors were observed in the animals:

Week of tumor observation		
	adenoma	carcinoma
1 mg/kg	1 at 93 wks 2 at 97 wks 3 at 100*, 101* wks	1 at 93 wks
3 mg/kg	1 at 96 wks 1 at 99 wks 5 at 100*, 101* wks	-----
10 mg/kg	5 at 100*, 101* wks	-----

\* terminal kill

DATA EVALUATION RECORD

## A. 3 Generation Reproductive Study

STUDY TYPE: Three generation reproductive study in the rat with teratology and behavioral data

CHEMICAL: Dinoseb, 2-sec butyl 4,6-dinitrophenol

TEST MATERIAL: Technical grade dinoseb; brown crystalline solid (batch # MM 2000-25) of 98.0% purity; blended with the basic powdered diet in a Morton '50E' batch mixer or a Gardner 3C double cone blender.

STUDY IDENTIFICATION:

a. Title: "Dinoseb Three Generation Reproductive Performance Study in the Rat (Dietary ) Hazleton Europe (#2006-50/19)

b. Laboratory: Hazleton Laboratories Europe Ltd.,  
Otley Road,  
Harrogate, HG3 1PY,  
England

c. Study Number: 2006-50/19

d. Study Date: August 1981

e. Study Director: L.F.H. Irvine, B.Sc.  
Department of Small Animal Toxicology

f. Caswell # 392DD; Accession # 259499-259506; EPA # 54299-Q (2)

CONCLUSIONS:1. Reproductive findings:

There is a consistent, compound-related decrease in body weight gain at the high dose in both adult males and females in the pre-mating period in all three generations, which continues in the treated males and females during mating, post-mating, etc. A systemic LEL is thus established at 10 mg/kg/day (LDT) based on depressed parental weight gain and the NOEL is 3 mg/kg/day. (The systemic LEL of 1 mg/kg/day (LDT) determined in the related 2 generation reproductive study is similar to the parental NOEL (3mg/kg/day) for systemic toxicity determined in this study).

The mean fetal weights were affected by dinoseb administration but with a high degree of variability. Decreased weights were observed or suggested in F<sub>0</sub>→F<sub>1b</sub>, F<sub>1</sub>→F<sub>2a</sub>, and F<sub>2</sub>→F<sub>3a</sub> littering groups with the F<sub>0</sub>→F<sub>1b</sub> pup weights diminished (combined sexes) at day 21 at all dose levels compared to controls. Since the pup weights at birth were similar, the decreased pup weight gains at day 21 indicate a reproductive effect of dinoseb related to the lactation period. Based on the findings for decreased pup weights, a reproductive LEL of 1 mg/kg/day is determined and a NOEL was not determined.

005432

## 2. Teratology

Dinoseb may be fetotoxic but the findings are variable. In the F<sub>0</sub>(F<sub>1b</sub>) pups there was an apparent dose-related increase in the overall skeletal defects ("minor" fetal defects) as compared with the control which was statistically significant at the high dose. For the F<sub>2</sub>(F<sub>3b</sub>) pups there was an apparent compound-related increase (not statistically significant) in the total number of "minor" skeletal defects due primarily to an increase (treatment-related) in sternebral and rib defects. However, the F<sub>2b</sub> pups did not appear to show any dose- or compound-related effects.

A NOEL cannot be established due to the small small number of dams utilized (9 to 10), the lack of litter incidence for fetal defects, and the variability of pre-implantation loss in the controls.

## 3. Behavioral data

No significant post-natal toxicity is ascribed to dinoseb administration at the doses studied in this assay in light of the small number of animals studied per group, the finding of a small weight change in only one group of rats (F<sub>1b</sub> males), and the lack of consistent, statistically significant effects.

With regards to the methodology for the study, a major deficiency in the study was the significant variability of the estimated dosages fed to the animals during the study as well as uncertainty regarding the analysis of the content of the fortified diet and the concentration of compound actually present in the diet. In addition, the report indicated the loss of food records for weeks 9 and 14-65 for both males and females which precludes an accurate estimate of the administered dose.

This study is classified Core Supplementary data.

METHODS:

A photocopy of the methods section has been appended. The following comments are noted:

1. It is noted on page 10 of the report that extreme fluctuations in temperature and humidity occurred, but a reason(s) is not given for the changes in the environment (see appended environmental charts). However, such changes must have been considerable since a number of deaths in both the males (2) and females (15) in the F<sub>2b</sub> generations were attributed to such extremes and pulmonary disease (e.g., congestion) appeared to be a frequent finding in the parents.
2. The stability and homogeneity of the test substance are of particular importance in a long-term test:
  - a. It is unclear as to whether the stability of technical Dinoseb itself was determined; this is critical since all the dietary mix was prepared from a single batch of test material shipped from the manufacturer, Dow Chemical Pacific Ltd( p. 12, Volume VI). 11, volume XI). Edgerton and Moseman (J.Agric.Chem.,26(2):425,1975) observed that their 2-sec-butyl-4,6-dinitrophenol (DNBP) liquid analytical standards significantly degraded(27% loss after 72 hrs) when stored in clear glass bottles. Were the internal standards of DNOC (4,6-dinitro-o-cresol) and DNBP used in the calibration curves and subsequent dietary stability tests (DNOC only) adequately controlled for chemical degradation over the life of the study?
  - b. Stability studies were performed for dinoseb in the feed (pages 141-143), for diet stored at room temperature (+ 22°C) or frozen (-20°C). Mean values were presented but no indication of the variability of the samples analyzed were presented, e.g., standard deviation .
3. The vagina, uterus, and seminal vesicles were not examined histologically as requested in both the 1978 Proposed and 1982 Final Guidelines for Toxicology Testing.
4. A smaller number of adult rats were examined per dose group in the F<sub>2</sub> males and females (10 each) for necropsy/histopathology than required by the EPA Guidelines (1978) which stated that for the F<sub>1</sub> adults (no third generation breeding was required in this test protocol) 10 males and 20 females were to be subjected to a complete gross necropsy and histopathology examination. The 1982 EPA Guidelines request full histopathology on the vagina, uterus, ovaries, testes, epididymus, seminal vesicles, prostate and target organ(s) for all high dose and control P<sub>1</sub> and F<sub>1</sub> (F<sub>0</sub> and F<sub>1</sub>, respectively, in the study under review) animals selected for mating.
5. Food consumption records were lost for weeks 9 and 14-65 for both males and females. The calculation for dinoseb intake (pages 110, 111) indicates that there was considerable variability in the doses administered based on the weekly diet intake and that the nominal dosages are only a crude estimate of the amount of test article the rats received. In the opinion of the reviewer, the variability in the dietary feed is unacceptable (a 15% variation would be acceptable). The following is an average of the weekly records reported:

005432

	Groups (average with range)		
	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
Males	0.961(.51-1.27)	2.934(1.54-4.06)	9.74(5.3-13.19)
Females	1.201(.73-1.67)	3.548(2.3-5.09)	11.478(7.06-17.84)

## RESULTS

### 1. Parental body weight changes : Table 1

Group mean parental body weight gains (g) are presented in Table 1 below. There is a consistent, compound-related decrease in body weight gain at the high dose in both males and females in the pre-mating period in all three generations [F<sub>0</sub> (325 and 144g=controls vs 218 and 111g=high dose, respectively); F<sub>1</sub> (419 and 204g=controls vs 357 and 185g=high dose, respectively); F<sub>2</sub> (358 and 195g=controls vs 310 and 180g=high dose, respectively)]. Although the mean weight gains fluctuate considerably, the males continue to exhibit a lower weight at the high dose than the controls during the period from mating to the study's completion [F<sub>1a</sub> (83g=control vs 61g=high dose); F<sub>2a</sub> (55g=control vs 21g=high dose); F<sub>3a</sub> (38g=control vs 25g=high dose)].

There continues to be a consistent but slight decrease in female weights during the gestation period in the a and b matings in all three generations at the high dose [F<sub>1a</sub> (108g=control vs 101g=high dose); F<sub>1b</sub> (136g=control vs 120g=high dose); F<sub>2a</sub> (136g=control vs 110g=high dose); F<sub>2b</sub> (113g=control vs 109g=high dose); F<sub>3a</sub> (104g=control vs 94g=high dose); F<sub>3b</sub> (121g=control vs 104g=high dose)]. This appears to be followed by a consistent "rebound" effect in weight at the high dose compared to the controls in all the lactation periods, i.e., F<sub>1a</sub> (-12g=control vs +2g=high dose); F<sub>1b</sub> (+3g=control vs +20g=high dose); F<sub>2a</sub> (+10g=control vs +13g=high dose); F<sub>2b</sub> (-10g=control vs +8g=high dose); F<sub>3a</sub> (+2g=control vs +23g=high dose); F<sub>3b</sub> (+17g=control vs +22g=high dose). However, the explanation for this phenomenon is uncertain since the females were continued on the compound during the lactation period.

Table 1: Group mean parental body weight gains(g)

Period	0 mg/kg/day	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
<u>[F0]</u>				
Pre-mating(1-14wks)				
Males	325	308	313	218
Females	144	132	132	111
<u>(F1a)</u>				
Gestation(0-21 days)				
Females	108	114	107	101
Lactation(1-21 days)				
Females	-12	-1	-2	+2
Mating-study end(21wk-29wk)				
Males	83	58	80	61
<u>(F1b)</u>				
Gestation(0-21 days)				
Females	136	137	131	120
Lactation(1-21 days)				
Females	+3	+10	+3	+20
<u>[F1]</u>				
Pre-mating(1-14wks)				
Males	419	377	402	357
Females	204	183	213	185
<u>(F2a)</u>				
Gestation(0-21 days)				
Females	136	122	139	110
Lactation(1-21 days)				
Females	+10	+18	+12	+13
Mating-study end(21-30wks)				
Males	55	47	33	21
<u>(F2b)</u>				
Gestation(0-21 days)				
Females	113	106	118	109
Lactation(1-21 days)				
Females	-10	-5	-5	+8

(Table continued on next page)

Table 1 (continued)

Period	0 mg/kg/day	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
<u>[F<sub>2</sub>]</u>				
Pre-mating(1-14wks)				
Males	358	324	367	310
Females	195	191	207	180
<u>(F<sub>3a</sub>)</u>				
Gestation(0-21 days)				
Females	104	105	103	94
Lactation(1-21 days)				
Females	+2	+22	+24	+23
Mating-study end(22-29wks)				
Males	38	61	60	25
<u>(F<sub>3b</sub>)</u>				
Gestation(0-21 days)				
Females	121	117	120	104
Lactation(1-21 days)				
Females	+ 17	+10	+6	+22

2. Food consumption (see Table 2 below)

No individual animal data were provided. Investigators indicated that the data were for the first 13 weeks of the F<sub>0</sub> generation and last 22 weeks of F<sub>2</sub> generation (F<sub>2a</sub> and F<sub>2b</sub>)---it is unclear which data relate to the respective generations. As indicated in Table 2, the apparent mean food consumption data for the females essentially doubled due to increased eating by the lactating dams and the pups eating food during later stages of the weaning period.

Table 2: Average of the group mean food consumption (g) data

Week #	0 mg/kg/day	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
1-13*				
Males	29.83	30.42	33.08	30.75
Females	32.17	29.67	32.25	31.75
66-87				
Males	29.13	29.59	31.41	32.04
Females†	54.55	59.73	55.50	52.36

\* week 10 data missing

† number of mean values from the littering/lactation period when female food intake doubles and pups also eating food during later stages

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### 3. Group Reproductive Indices: Table 3

Group mean reproductive indices are presented in Table 3 below (mean litter size is presented in Table 4). No significant effects were noted upon examination of the adult data for male and female fertility, and the gestation index--a measure of the number of pregnant females with live pups--for either F<sub>0</sub>, F<sub>1</sub> or F<sub>2</sub> generations at any dose level in either mating (a,b). Further, there was no indication of any real compound-related decrease in fetal viability in any generation as measured by the live birth index (measure of pup viability at birth), viability index (pup viability at day 4), or lactation index (pup viability at weaning), although there is a slight decrease in the viability indices at the mid dose in the F<sub>3a</sub> and F<sub>3b</sub> litters.

There were no significant differences between the control and treated group mean litter sizes, although the control littering groups were somewhat smaller and more variable in the F<sub>2</sub>→F<sub>3a,b</sub> groups as compared to the two other generations [F<sub>2</sub>→F<sub>3a</sub> = 10.3(2.48), F<sub>2</sub>→F<sub>3b</sub> = 10.5(3.53) versus F<sub>0</sub>→F<sub>1a</sub> = 13.2(2.28), F<sub>0</sub>→F<sub>1b</sub> = 12.7(2.29); F<sub>1</sub>→F<sub>2a</sub> = 13.1(2.12), F<sub>1</sub>→F<sub>2b</sub> = 13.8 (1.97)].

### 4. Mean Fetal Indices (Littering group): Table 4

Examination of the mean fetal indices (Table 4) indicates that a number of parameters were affected, although of a somewhat inconsistent nature.

The F<sub>0</sub>→F<sub>1a</sub> data do not suggest any significant compound effects on fetal weight at birth (although the per cent increase in pup weights for the treated groups did fluctuate some at both days 4 and 21 post-partum) but decreased weight gains occur in all the other littering groups except for the last mating (F<sub>2</sub>→F<sub>3b</sub>). F<sub>0</sub>→F<sub>1b</sub> pup weights were diminished (combined sexes unless otherwise indicated) at day 21 at all dose levels compared to controls (41.2g/cont., 37.7g/low, 37.8g/mid, 37.0g/high) and the per cent weight increases (524.1/cont., 480.0/low, 455.9/mid, 460.6/high) were statistically significantly (ss) lower at all dose levels (p<0.05). This is reflected by the lower pup weight gains seen in the individual sexes at day 21 and indicates an effect of dinoseb on the pups during lactation since the pup weights at birth were similar.

Although not statistically significant, similar effects to those in the F<sub>0</sub>→F<sub>1b</sub> littering groups were noted in the F<sub>1</sub>→F<sub>2a</sub> pup weights (g) at day 21 (see Table 4) again suggesting a compound-related effect during the lactation period and which could relate to an inadequate milk supply (hormonal or systemic effect in the dams) or to a direct toxic effect of the dinoseb on the pups.

The toxicity of dinoseb in the F<sub>2</sub>→F<sub>3a</sub> littering group is different in that a statistically significant decrease in pup weight (g) at day 1 at all dose levels is observed as compared to control values (6.5g/cont., 6.0g/low, 5.8g/mid, 5.8g/high; p<0.05 or p<0.01). Diminished weight gains are seen at day 4 [9.5g/cont., 8.4g/low, 8.2g/mid (s.s.), 8.1g/high] and at day 21 (weaning) [36.5g/cont., 32.9g/low, 32.6g/mid, 30.5g/high (s.s.)]. This again indicates a definite reproductive toxicity occurring initially during gestation as inhibition of fetal growth and continuing during the lactation period.

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Table 3: Group Mean Reproductive Indices

Parameter	0 mg/kg/day	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
Male Fertility( $F_0$ )†	100.0	100.0	100.0	100.0
Female Fertility( $F_0$ )†	100.0 <sup>¶</sup>	92.0 <sup>¶</sup>	100.0	98.0 <sup>¶</sup>
$F_0 \rightarrow F_{1a}$				
# animals mated	23	25	24	24
# animals not mated	2	0	1	1
# pregnancies	23	24	24	24
mating index	100.0	96.2	92.3	100.0
fecundity index	100.0	96.0	100.0	100.0
gestation index	100.0	100.0	100.0	100.0
live birth index	98.7	93.6	93.7	97.2
viability index	95.0	98.2	97.3	96.4
lactation index	91.9	92.3	94.8	87.7
$F_0 \rightarrow F_{1b}$				
# animals mated	24	25	24	25
# animals not mated	1	0	1	0
# pregnancies	24	22	24	24
mating index	100.0	100.0	100.0	100.0
fecundity index	100.0	88.0	100.0	96.0
gestation index	95.8 <sup>¶</sup>	100.0	100.0	100.0
live birth index	93.2	100.0	100.0	100.0
viability index	97.6	100.0	100.0	100.0
lactation index	91.3	93.5	86.8	91.1
Male Fertility( $F_1$ )†	100.0	100.0	100.0	100.0
Female Fertility( $F_1$ )†	94.0 <sup>¶</sup>	89.4 <sup>¶</sup>	97.9.0 <sup>¶</sup>	93.8 <sup>¶</sup>
$F_1 \rightarrow F_{2a}$				
# animals mated	25	22	22	25
# animals not mated	0	3	3	0
# pregnancies	25	20	22	24
mating index	100.0	90.9	100.0	86.2
fecundity index	100.0	90.9	100.0	96.0
gestation index	100.0	100.0	100.0	95.7
live birth index	98.8	99.2	99.3	99.2
viability index	96.0	96.9	94.9	95.7
lactation index	97.1	98.0	95.7	95.5
$F_1 \rightarrow F_{2b}$				
# animals mated	25	25	25	23
# animals not mated	0	0	0	1
# pregnancies	22	22	24	21
mating index	92.0	76.7	86.2	88.5
fecundity index	88.0	88.0	96.0	91.3
gestation index	100.0	100.0	100.0	100.0
live birth index	99.5	94.3	97.3	100.0
viability index	98.4	100.0	94.0	99.3
lactation index	97.9	99.4	97.5	99.3

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Table 3 (continued);

Parameter	0 mg/kg/day	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
Male Fertility(F <sub>2</sub> )†	100.0	100.0	100.0	100.0
Female Fertility(F <sub>2</sub> )†	96.0	98.0%	100.0	100.0
<u>F<sub>2</sub>→F<sub>3a</sub></u>				
# animals mated	24	25	25	24
# animals not mated	1	0	0	1
# pregnancies	23	24	25	24
mating index	100.0	100.0	100.0	92.0
fecundity index	95.8	96.0	100.0	100.0
gestation index	100.0	100.0	96.0	100.0
live birth index	99.2	100.0	97.0	99.2
viability index	91.9	94.2	78.4	86.8
lactation index	97.7	89.6	98.7	96.4
<u>F<sub>2</sub>→F<sub>3b</sub></u>				
# animals mated	25	24	24	25
# animals not mated	0	0	0	0
# pregnancies	24	24	24	25
mating index	100.0	100.0	95.8	95.8
fecundity index	96.0	100.0	100.0	100.0
gestation index	100.0	100.0	100.0	93.3
live birth index	91.9	99.3	97.1	98.6
viability index	100.0	100.0	88.7	92.4
lactation index	96.0	97.2	96.0	91.0

† combined F<sub>a</sub> and F<sub>b</sub> generations; %incorrectly calculated in report

F<sub>2</sub>→F<sub>3b</sub> data indicate an increase in preweaning loss (%) at the mid- and high dose (statistically significant) as compared to the controls (11.8/control, 17.3/mid, 17.0/high), but no other effects on fetal indices were noted for this mating. An increase is also suggested in the F<sub>2</sub>→F<sub>3a</sub> groups (all doses), although no statistically significant changes were reported ( 11.0/control, 15.6/low, 25.0/mid, 17.0/high).

##### 5. Reproductive Organ Weights: Table 5

Gonadal organ weights and organ-to-body weight ratios are presented below in Table 5 for both males and females from the F<sub>2</sub> adults and F<sub>3b</sub> pups since these were the only data provided by the investigators. No consistent pattern of change can be observed although the organ-to-body weight ratio in the adult treated males is higher for either gonad (.314/control, .380/high:left; .326/control, .389/high:right) or for the total weight ratio (.640/control, .769/high)--an effect which may be related to the decreased body weight of the male rats in the high dose group( see parental body weight discussion).

Table 4: Mean Fetal Indices (Littering group)

	0 mg/kg/day	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
<u>F0--&gt;F1a</u>				
% pre-weaning loss	13.8	15.2	9.0	17.8
ratio males:females	1:1.10	1:0.94	1:0.88	1:0.97
mean litter size <sup>a</sup>	13.2(2.28)	12.4(2.84)	12.5(3.18)	11.9(3.50)
pup wt. day 1(g)†	6.4	6.6	6.3	6.3
pup wt. day 4(g)/	8.8/37.5	8.8/43.9	8.9/41.3	8.7/38.1
% wt. increase				
pup wt. day 21(g)/	35.5/454.7	38.9/489.4	37.1/488.9	32.9/422.2
% wt. increase over weaning period				
male pup wt. day 21(g)	35.9	39.8	37.4	32.9
female pup wt. day 21(g)	35.5	39.5	36.8	32.3
<u>F0--&gt;F1b</u>				
% pre-weaning loss	16.9	8.7	13.2	8.9
ratio males:females	1:1.19	1:1.07	1:1.02	1:0.91
mean litter size <sup>a</sup>	12.7(2.29)	13.2(1.96)	12.6(3.26)	12.1(1.66)
pup wt. day 1(g)†	6.6	6.5	6.8	6.6
pup wt. day 4(g)/	8.7/31.8	9.2/41.5	9.2/35.3	8.8/33.3
% wt. increase				
pup wt. day 21(g)/	41.2/524.1	37.7/480.0*	37.8/455.9*	37.0/460.6*
% wt. increase over weaning period				
male pup wt. day 21(g)	42.0	38.8	39.1	38.2
female pup wt. day 21(g)	40.5	36.4	36.8	36.0
<u>F1--&gt;F2a</u>				
% pre-weaning loss	8.0	5.7	9.8	9.3
ratio males:females	1:0.89	1:1.10	1:1.14	1:1.05
mean litter size <sup>a</sup>	13.1(2.12)	13.1(3.32)	13.5(2.44)	12.7(2.76)
pup wt. day 1(g)†	6.2	6.3	6.0	5.8
pup wt. day 4(g)/	8.1/30.6	8.0/27.0	8.4/40.0	7.7/32.8
% wt. increase				
pup wt. day 21(g)/	36.9/495.2	36.5/471.4	33.9/465.0	33.9/484.5
% wt. increase over weaning period				
male pup wt. day 21(g)	37.4	37.1	34.2	34.5
female pup wt. day 21(g)	36.7	35.3	33.4	33.3
<u>F1--&gt;F2b</u>				
% pre-weaning loss	4.1	6.3	10.9	1.3
ratio males:females	1:1.01	1:1.14	1:0.97	1:1.17
mean litter size <sup>a</sup>	13.8(1.97)	13.4(3.07)	14.7(2.25)	12.8(1.11)
pup wt. day 1(g)†	5.5	6.2	5.8	5.9
pup wt. day 4(g)/	9.1/65.5	9.8/58.1	9.0/55.2	9.0/52.5
% wt. increase				
pup wt. day 21(g)/	32.8/496.4	36.6/490.3	32.2/455.2	32.2/445.8
% wt. increase over weaning period				
male pup wt. day 21(g)	33.5	37.8	32.9	32.8
female pup wt. day 21(g)	32.3	35.7	31.3	31.8

\* significantly different from controls ( $p < 0.05$ ; t test)

<sup>a</sup> number of pups born per dam (S.D.)

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Table 4 (continued)

<u>F2--&gt;F3a</u>	<u>0 mg/kg/day</u>	<u>1 mg/kg/day</u>	<u>3 mg/kg/day</u>	<u>10 mg/kg/day</u>
% pre-weaning loss	11.0	15.6	25.0	17.0
ratio males:females	1:0.99	1:0.76	1:1.00	1:0.79
mean litter size <sup>a</sup>	10.3(2.48)	11.5(2.45)	12.3(2.18)	10.8(2.04)
pup wt. day 1(g)†	6.5	6.0*	5.8*	5.8**
pup wt. day 4(g)/	9.5/46.2	8.4/40.0	8.2*/41.4	8.1**/39.7
% wt. increase				
pup wt. day 21(g)/	36.5/461.5	32.9/448.3	32.6/462.1	30.5**/425.9
% wt. increase over weaning period				
male pup wt. day 21(g)	37.3	32.9	33.4	31.0
female pup wt. day 21(g)	35.9	32.4	31.8	29.9
<u>F2--&gt;F3b</u>				
% pre-weaning loss	11.8	3.5	17.3	17.0***
ratio males:females	1:0.82	1:1.01	1:0.93	1:1.14
mean litter size <sup>a</sup>	10.5(3.53)	11.8(3.35)	12.4(2.62)	10.5(3.16)
pup wt. day 1(g)†	5.8	6.2	5.8	6.1
pup wt. day 4(g)/	8.5/46.6	8.9/43.5	8.1/39.7	8.5/39.3
% wt. increase				
pup wt. day 21(g)/	33.4/475.9	33.0/432.3	30.6/427.6	35.2/477.0
% wt. increase over weaning period				
male pup wt. day 21(g)	34.3	33.5	31.1	36.0
female pup wt. day 21(g)	32.0	32.4	30.0	34.7

† from animals giving birth to live pups (mean of both sexes)

\* significantly lower than the control group ( $p < 0.05$ : Wilcoxon's test)\*\* significantly lower than the control group ( $p < 0.01$ : Wilcoxon's test)\*\*\* significantly higher than the controls ( $p < 0.05$ : Fisher's test)<sup>a</sup> number of pups per dam (S.D.)

Table 5 : Organ weights/organ-to-body weight ratios: reproductive organs

<u>F<sub>2</sub>→F<sub>3</sub></u>			
<u>F<sub>2</sub> adults</u>	<u>Gonad mean wt(g), ( S.D.)/organ-to-b.wt. ratio(%), (S.D.)</u>		
	<u>Left</u>	<u>Right</u>	<u>Total</u>
1M	1.6(.481)/.314(.083)	1.7(.520)/.326(.096)	3.3(.982)/.640(.174)
1F	.053(.012)/.017(.003)	.049(.008)/.016(.002)	.102(.019)/.033(.004)
2M	1.6(.145)/.329(.047)	1.7(.221)/.341(.054)	3.3(.352)/.670(.098)
2F	.057(.015)/.019(.006)	.054(.009)/.018(.004)	.111(.021)/.037(.009)
3M	1.7(.232)/.313(.047)	1.6(.284)/.311(.055)	3.3(.500)/.623(.099)
3F	.047(.013)/.016(.004)	.048(.009)/.016(.003)	.095(.019)/.032(.007)
4M	1.55(.246)/.380(.065)	1.58(.187)/.389(.063)	3.13(.416)/.769(.124)
4F	.050(.015)/.018(.007)	.054(.017)/.019(.008)	.104(.031)/.037(.015)
<u>F<sub>3b</sub> progeny</u>			
1M	.430(.066)/.516(.048)	.430(.066)/.516(.048)	.861(.133)/1.0(.097)
1F	.012(.003)/.016(.003)	.013(.003)/.018(.004)	.025(.004)/.034(.002)
2M	.291(.117)/.395(.083)	.300(.118)/.408(.089)	.591(.234)/.803(.172)
2F	.015(.002)/.020(.004)	.014(.003)/.019(.007)	.028(.005)/.039(.011)
3M	.380(.084)/.661(.314)	.400(.071)/.686(.289)	.780(.148)/1.3(.601)
3F	.014(.005)/.018(.005)	.012(.003)/.016(.003)	.025(.008)/.034(.008)
4M	.323(.056)/.425(.056)	.349(.067)/.460(.083)	.672(.116)/.885(.123)
4F	.017(.007)/.026(.008)	.017(.006)/.025(.006)	.034(.012)/.051(.014)

1= 0 mg/kg/day, 2= 1 mg/kg/day, 3= 3 mg/kg/day, 4= 10 mg/kg/day

005432

Table 5A-Caesarian Data: F<sub>0</sub>→F<sub>1b</sub>

Parameters	G1	G2	G3	G4
<u>General</u>				
# pregnancies(%)	10/10(100)	9/10(90)	10/10(100)	9/10(90)
# corpora lutea(c.l.)	143	127	145	114
functional c.l.per dam	14.3	15.9	14.5	12.7*
# implantations	139	118	133	107
# Implants./preg- nant dam	13.9	13.1 <sup>+</sup>	13.3	11.9
% pre-implant. loss	2.8	7.1	8.3**	6.1
<u>Fetal data</u>				
#/# per pregnant dam	134(13.4)	114(12.7) <sup>+</sup>	127(12.7)	105(11.7)
% of implantation	96.4	96.6	95.5	98.1
Early deaths(§)/mean per # pregnant dam	5/0.5	4/0.44 <sup>+</sup>	6/0.6	2/0.22
Early deaths(% im- plant.)/#late deaths	3.6/0	3.4/0	4.5/0	1.9/0
Total # intrauterine (i.u.) deaths/mean # per pregnant dam	5/0.5	4/0.44 <sup>+</sup>	6/0.6	2/0.22
# males/# females	69/65	61/53	72/55	48/57
male:female ratio	1.0:0.94	1.0:0.87	1.0:0.76	1.0:1.19
Mean litter wt(g): live fetuses	75.5	75.3	70.1 <sup>a</sup>	65.3
Mean live fetal wts (g) [male/female]	5.7/5.6	5.4/5.2	5.8 <sup>a</sup> /5.5 <sup>a</sup>	5.8/5.5
Overall fetal wt. mean (live fetal)	5.7	5.3	5.7 <sup>a</sup>	5.6
Crown/rump length(mm)	45.6	42.9	44.4 <sup>a</sup>	43.1
<u>External and visceral effects</u>				
"Minor" defects (#fetuses)/% fe- tuses examined	12/9.0	13/11.5	11/8.7	10/9.3
<u>Skeletal defects</u>				
# fetuses examd.	89	77	86	71
# "Minor defects"/ % fetuses examd.	9/10.1	11/14.3	20/23.3	24/33.8**
<u>Variantst</u>				
# Fetuses/% of fetuses examd.	14/15.7	31/40.3	14/16.3	11/15.5

<sup>+</sup> number stated in text incorrectly; \*statistically significant at p<0.05 (Wilcoxon test); \*\*statistically significant at p<0.05 (Fisher's test); <sup>a</sup> 8 litters; <sup>+</sup> as defined by the investigators G1=0 mg/kg/day, G2=1 mg/kg/day, G3=3 mg/kg/day, G4= 10 mg/kg/day

Table 5B-Caesarian Data: F<sub>1</sub>→F<sub>2b</sub>

Parameters	G1	G2	G3	G4
<u>General</u>				
# pregnancies(%)	8/10(80)	9/10(90)	9/10(90)	9/10(90)
# corpora lutea(c.l.)	121	119	145	123
functional c.l.per dam	15.1	13.2	16.1	13.7
# implantations	116	95	138	118
# Implants./preg-	14.5	10.6	15.0	13.1
nant dam				
% pre-implant. loss	4.1	20.2*	4.8	4.1
<u>Fetal data</u>				
#/# per pregnant dam	115/14.4	93/10.3	138/15.3	118/13.1
% of implantation	99.1	97.9	100.0	100.0
Early deaths(=)/mean	1/0.1	1/0.1	0/0.0	0/0.0
per # pregnant dams				
Early deaths(% im-	0.9/0.0	1.1/0.0	0.0/0.0	0.0/0.0
plant.)/late deaths				
Total # intrauterine	1/0.1	2/0.2	0/0.0	0/0.0
(i.u.) deaths/mean #				
per pregnant dam				
# males/# females	63/52	51/42	61/77	54/64
male:female ratio	1:0.83	1:0.82	1:1.26	1:1.19
Mean litter wt(g):	74.1	55.5	82.0	69.2
live fetuses				
Mean live fetal wts	5.3/5.1	5.3/4.8	5.6/5.2	5.4/5.2
(g) (male/female)				
Overall fetal wt.	5.2	5.2	5.4	5.3
Mean (live fetal)	43.6	44.5	44.4	44.1
crown/rump length(mm)				
<u>External and visceral effects</u>				
"Minor"† defects	10/8.7	6/6.5	10/7.2	14/11.9
(#fetuses)/% fe-				
tuses examined				
"Major"† defects, #	0/0.0	1/1.1	3/2.2	1/0.8
fetuses/% fets. examd.				
<u>Skeletal defects</u>				
# fetuses examd.	78	61	94	91
# "Minor defects"/	26/33.3	12/19.7	19/20.2	24/29.6
% fetuses examd.				
<u>Variantst</u>				
# Fetuses/% of	34/43.6	18/29.5	36/38.3	30/37.0
fetuses examd.				

† As defined by investigators; \* significant at p<0.01 level using Chi Square (reviewer's calculation)

Table 5C-Caesarian Data: F<sub>2</sub>→F<sub>3b</sub>

Parameters	G1	G2	G3	G4
<u>General</u>				
# pregnancies(%)	11/12(91.7)	12/12(100)	10/10(100)	10/10(100)
# corpora lutea(c.l.)	163	160	159	129
functional c.l.per dam	14.8	13.3	15.9	12.9
# implantations	135	158	144	120
# Implants./preg- nant dam	12.3	13.2	14.4	12.0
# Pre-implant. loss	17.2	1.3	9.4	7.0
<u>Fetal data</u>				
#/# per pregnant dam	133/12.1	157/13.1	143/14.3	120/12.0
% of implantation	98.5	99.4	99.3	100.0
Early deaths(§)/mean	2/0.2	1/0.1	1/0.1	0/0.0
per # pregnant dams				
Early deaths(% im- plant.)/#late deaths	1.5/0.0	0.6/0.0	0.7/0.0	0.0/0.0
Total # intrauterine (i.u.) deaths/mean # per pregnant dam	2/0.2	1/0.1	1/0.1	0/0.0
# males/# females	71/62	80/77	65/78	64/56
male:female ratio	1:0.87	1:0.96	1:1.20	1:0.88
Mean litter wt(g): live fetuses	64.7	69.6	72.4	59.7
Mean live fetal wts (g) [male/female]	5.5/5.2	5.3/5.1	5.3/5.0	5.1/4.9
Overall fetal wt.	5.3	5.2	5.2	5.0*
Mean (live fetal) crown/rump length(mm)	44.7	44.6	44.3	44.0
<u>External and visceral effects</u>				
"minor" defects (#fetuses)/% fe- tuses examined	11/8.3	9/5.7	9/6.3	6/5.0
<u>Skeletal defects</u>				
# fetuses examd.	93	104	99	81
# "Minor defects"/ % fetuses examd.	22/23.7	36/34.6	38/38.4	32/39.5
<u>Variantst</u>				
# Fetuses/% of fetuses examd.	41/44.1	75/72.1	75/75.8*	66/81.5*

t as defined by the investigators; \* statistically significant at  $p < 0.05$  (Wilcoxon's test)

Table 5D: Nature and incidence of fetal defects\*(Caesarian group)

F0--&gt;F1b (p.47, vol. VII)

## SKELETAL DEFECTS

<u>sternbrae</u>	<u>G1</u>	<u>G2</u>	<u>G3</u>	<u>G4</u>
sternbrae 1,3,4, two distinct pts. ossific.	1			
" " 5, two distinct pts. ossific.	1	2	1	4
" " 1,2,3,4,5, two distinct pts. ossific.				1
" " 3 fused				1
" " 3,4,5, asymmetric ossific.			1	1
" " 2 malformed		1		
" " 1 incompletely ossified		1		
" " 5 not ossified	1			
<u>ribs</u>				
extra pair ribs	(1)	1	3	9
single extra rib	1	2	3(1)	2
<u>vertebrae</u>				
vertebrae centra bipartite usually 10-13	5(2)	6	13	14(5)
Subtotal:	9(12)	13	21(22)	32(37)
<u>variants</u>				
forelimb phalanges incompletely/not ossified	1	6		
hindlimb phalanges incompletely/not ossified	14	31(2)	14	11(8)
Subtotal:	15	37(39)	14	11(8)

F1--&gt;F2b (p.47, vol. VIII)

## VISCERAL DEFECTS

<u>urogenital system</u>	<u>G1</u>	<u>G2</u>	<u>G3</u>	<u>G4</u>
Minor:				
increased cavitation in renal pelvis	1			
both ureters dilated	2	1	3	7
left ureter dilated		1	5	3
right ureter dilated	7		2	4
Subtotal:	10	2	10	14
Major:				
hydronephrosis of right kidney		1		
hydronephrosis of both kidneys			3	1

(continued on next page)

## SKELETAL DEFECTS

<u>skull(minor)</u>	<u>G1</u>	<u>G2</u>	<u>G3</u>	<u>G4</u>
frontal bones incompletely ossified	2		1	
frontal bones fissured			3	
parietal bones incompletely ossified	7	1	2	1
interparietal bones incompletely ossified	7			
occipital bones incompletely ossified	3			
nasal bones incompletely ossified	1			
Subtotal:	20	1	6	1
<u>sternebrae (minor)</u>				
5th sternebra bipartite	2	5	1	1
" " " incompletely/not ossified		9	5	7
6th " " " " " "		4		1
2nd " " " " " "		1	1	1
6th sternebra has bony projection		1		
Subtotal:	2	19	8	10
<u>ribs (minor)</u>				
extra pair ribs	1	11	3	1
single extra rib	4	3	8	6
13th rib/ribs vestigial			1	
12th rib vestigial			1	
Subtotal:	5	14	13	7
<u>vertebrae (minor)</u>				
thoracic vrtbr. in region 10-13 bipartite	12	6	12	6
" " " 11 not ossified		1		
" " " 3 bipartite			1	
lumbar vertebra 1 bipartite	1		1	
Subtotal:	13	7	14	6
<u>limbs (minor)</u>				
metacarpals incompletely/not ossified	2	8		7
metatarsals " " " "	6	2	8	14
Subtotal:	8	10	8	21
:	48	51	49	45

## 6. Caesarian Data

Tables 5A-C present a summary of teratology data for the  $F_0(F_{1b})$ ,  $F_1(F_{2b})$ , and  $F_2(F_{3b})$  generation parental and fetal data, and Table 5D presents the nature and incidence of fetal defects of possible concern. The following discussion refers to these tables. [Note: not statistically significant=nss].

The maternal data indicates some compound-related effects, primarily in the  $F_0$  data. A statistically significant ( $p < 0.05$ ) decrease in the mean number of functional corpora lutea (12.7 vs 14.3 in control) at the high dose was noted resulting in a lower (nss) number of implants per pregnant dam (11.9 vs 13.9 in control). There were a number of significant changes in the pre-implantation losses in all generations but their meaning is uncertain in light of the large variation in the controls (ranged from 2.8 to 17.2%). There was a higher percentage of pre-implantation losses in all dose groups ( $F_0$ ) as compared to the controls, with the mid-dose being statistically significant (2.8=cont., 7.1=low, 8.3=mid, 6.1=high). In the  $F_1$  data there was a large increase at the low dose in percentage pre-implantation loss (20.2 vs 4.1 in the control) while there appeared to be a diminution (nss) in the % pre-implantation loss in all dose groups of the  $F_2$  generation as compared to the controls (17.2=cont., 1.3=low, 9.4=mid, 7.0=high). As indicated above, the control value was on the upper end of the control range for pre-implantation losses so that the diminution may not be a real effect.

Somewhat erratic effects in the general fetal data are noted in all three generations. In the  $F_1$  fetuses an apparent decrease in the number of early deaths (% implantations) (1.9 vs 3.6 in controls, nss) is noted in the high dose group, an effect which is also suggested in the  $F_2$  and  $F_3$  fetuses (0.0 vs 0.9 in control, 0.0 vs 1.5 in control). While male:female ratios appear to be generally increased in the treated dams of all three generations at the higher doses ( $F_1$ : 1:0.94=contr., 1:1.19=high;  $F_2$ : 1:0.83=cont., 1:1.26=mid, 1:1.19=high;  $F_3$ : 1:0.87=cont., 1:1.20=mid), the significance of this is unclear. In the  $F_3$  pups there is a small but consistently lower mean fetal weight (g) which is statistically significantly lower ( $p < 0.05$ ) at the high dose (5.3g=cont., 5.2g=low and mid, 5.0g=high). There was no consistent effect of dose on fetal length as measured by the mean crown/rump length in the  $F_1$ ,  $F_2$  or  $F_3$  pups.

External, visceral, and skeletal defects are presented in summary form in Tables 5 A-C and the specific nature and incidence of the fetal defects are presented in Table 5 D. Possible compound- and/or dose-related toxicity was observed for all offspring of the  $F_0$  and  $F_2$  generations.

In the  $F_{1b}$  pups there was a suggestion of a dose-related increase in the overall skeletal defects (% "minor" fetal defects) as compared with the control which was statistically significant at the high dose (10.1=cont., 14.3=low, 23.3=mid, 33.8=high), while the % "variants" was increased (nss) only in the low dose group (15.7=cont. vs 40.3=low). (It should be noted that the authors stated that fetuses showing more than one defect were included only once in the overall calculation of defective fetuses). Minor skeletal defects were defined as common deviations from normal, whereas skeletal variants were defined as incompletely or non-ossified phalanges.

In the  $F_{2b}$  pups there was a small number of "major" visceral defects, i.e., hydronephrosis of right or both kidneys, reported in the treated animals (0=contr.,

1=low, 3=mid, 1=high). These may not be significant since they may relate to an artifact in the processing of the fetuses (Woo and Hoar, 1982; Teratology 25:82) or may be an effect which is reversible.

For the F<sub>3b</sub> pups there was an apparent compound-related increase(nss) in the total number of "minor" skeletal defects which, when examined in detail, resulted primarily from an increase (treatment-related) in sternal and rib defects. Variants (forelimb or hindlimb phalanges incompletely/not ossified) were consistently increased in a compound-related fashion (statistically significant at mid- and high-dose levels). Without the individual litter data these findings cannot be verified.

Dinoseb appears fetotoxic, a finding not surprising in light of its structural analogy to 2,4-dinitrophenol, a metabolic poison, or to Karathane( a mixture of 2,4-dinitro-6-octylphenyl crotonate and 2,6-dinitro-4-octylphenyl crotonate) which is teratogenic in rabbits (oral administration) at 3mg/kg/day (memo of Q. Bui dated 4/1/85).

While the findings of fetotoxicity are of qualitative importance, there are a number of reasons why a NOEL cannot be established in this study. First, the small number of dams utilized (9 to 10) precludes the determination of fetotoxicity with any statistical confidence. Furthermore, the investigators did not present litter incidence for fetal defects. Finally, the pre-implantation loss is quite variable in the controls, making interpretation of the findings uncertain.

#### 7. Behavioral Data

Postnatal data for F<sub>1</sub> and F<sub>2</sub> pups (taken from the b matings) are presented in Table 6 below. Mean body weights (g) were slightly but consistently lower in the F<sub>1b</sub> males at every period of weight measurement (weeks 1, 5, 10, 14) for all dose levels as compared to the controls, with the weights ranging from 83-95% of control.

F<sub>1</sub> pups did not appear to be affected in their ability to remain on the rotating rod at day 14 post-partum but there was a suggestion of a dose-related effect in the mean trial time values (seconds) for the F<sub>2</sub> pups (5.2/cont, 4.8/low, 4.3/mid, 4.0/high). However, for rotating rod trial time at 5-6 weeks there was no consistent effect seen for either F<sub>1</sub> or F<sub>2</sub> male or female animals, although the F<sub>2</sub> male data suggest a dose-related diminished ability to maintain balance (42.6/cont., 39.8/low, 31.1/mid, 28.5/high). A "behavioral" effect of dinoseb on the F<sub>2</sub> males is further suggested by the consistent treatment-related decrease in mean trial time(seconds) on the spiral at 13 weeks in 2 sets of trials (mean trial: 8.4/cont., 7.3/low, 6.3/mid, 6.3/high; trial 4: 7.3/cont., 6.3/low, 5.6/mid, 5.9/high).

Auditory startle response, visual placing response or observation of gait did not appear to be affected by dinoseb administration for any group at the time measured (5-6 weeks).

Table 6: Behavioral Data

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Mean Body weights(g)	week 1	week 5	week 10	week 14
F <sub>1</sub> (males)				
G1	90	342	466	510
G2	78	295	439	481
G3	82	298	426	487
G4	75	288	435	476
F <sub>2</sub> (males)				
G1	62	199	355	411
G2	70	206	335	384
G3	80	231	379	433
G4	76	212	349	393
F <sub>1</sub> (females)				
G1	81	233	264	285
G2	81	218	248	270
G3	70	189	245	266
G4	72	198	257	283
F <sub>2</sub> (females)				
G1	64	159	234	244
G2	69	172	210	249
G3	73	172	210	249
G4	77	190	210	250

Rotating rod-day 14 post-partum

	Mean trial time (seconds)/litter (S.D.)
F <sub>1</sub> pups	
G1	3.0(0.58)
G2	3.7(2.40)
G3	3.1(0.98)
G4	3.2(1.57)

F <sub>2</sub> pups	
G1	5.2(1.89)
G2	4.8(1.59)
G3	4.3(1.49)
G4	4.0(1.34)

	<u>Auditory (startle) response</u>	<u>Visual placing response</u>	<u>Observation of gait</u>	<u>Rotating rod trial time (secs): group mean(S.D.)</u>
<u>Males</u>				
F <sub>1</sub> (5-6 wks)				7.2(5.79)
G1	10/10*	10/10*	10/10*	20.7(19.04)
G2	8/8	8/8	8/8	8.0(2.45)
G3	5/5	5/5	5/5	11.9(8.58)
G4	9/9	9/9	9/9	

(continued next page)

Table 6 (continued)

	Auditory (startle) response	Visual placing response	Observation of gait	Rotating rod trial time (secs): group mean(S.D.)
<u>(males)</u>				
F <sub>2</sub> (5-6wks)				42.6(18.89)
G1	10/10	10/10	10/10	39.8(25.80)
G2	8/8	8/8	8/8	31.1(23.91)
G3	10/10	10/10	10/10	28.5(26.24)
G4	8/8	8/8	8/8	
<u>Females</u>				
F <sub>1</sub> (5-6wks)				20.3(21.54)
G1	10/10	10/10	10/10	40.1(23.48)
G2	8/8	8/8	8/8	37.2(14.32)
G3	6/6	6/6	6/6	21.4(21.31)
G4	10/10	10/10	10/10	
F <sub>2</sub> (" ")				35.9(26.04)
G1	10/10	10/10	10/10	41.5(25.70)
G2	8/8	8/8	8/8	40.7(23.19)
G3	10/10	10/10	10/10	33.3(25.20)
G4	8/8	8/8	8/8	

Time balanced on spiral  
Mean trial time (3 trials) (seconds) Trial 4 (24 hrs later)

<u>Males</u>		
F <sub>1</sub> (aprox.14wks)		6.4(4.30)
G1	5.3(1.32)	9.6(5.26)
G2	7.3(1.34)	4.0(1.00)
G3	4.4(1.22)	6.7(3.91)
G4	6.3(2.03)	
<u>Females</u>		
F <sub>1</sub> (aprox.14wks)		8.4(3.57)
G1	6.3(2.28)	11.4(4.21)
G2	9.8(3.39)	11.2(3.06)
G3	10.8(2.67)	8.7(4.08)
G4	7.4(1.37)	
F <sub>2</sub> (" 13wks)		7.0(4.71)
G1	8.5(4.00)	11.3(5.85)
G2	11.1(5.22)	8.1(3.87)
G3	9.1(3.33)	8.5(5.71)
G4	9.0(2.72)	

\*+ = normal response

G1= 0 mg/kg/day, G2= 1 mg/kg/day, G3= 3 mg/kg/day, G4= 10 mg/kg/day

## 7. Behavioral Data (continued)

Examination of gross necropsy data for both F<sub>1</sub> and F<sub>2</sub> pups indicated that the pulmonary system may be affected by dinoseb administration, particularly in the males at the mid and high dose:

Group's sex (#)	0 mg/kg/day		1 mg/kg/day		3 mg/kg/day		10 mg/kg/day	
	males	females	males	females	males	females	males	females
F <sub>1</sub>	(10)	(10)	(8)	(8)	(5)	(6)	(9)	(10)
pulmonary congestion	1(10%)	—	2(25%)	1(13%)	1(20%)	2(33%)	4(44%)	—
pulmonary sub-pleural grey foci	1(10%)	2(20%)	2(25%)	1(13%)	1(20%)	2(33%)	4(44%)	1(10%)
F <sub>2</sub>								
pulmonary sub-pleural grey foci	2(20%)	2(20%)	2(25%)	—	4(40%)	3(30%)	5(63%)	2(25%)

## 8. Necropsy Data (other than behavioral animals): Table 7

Yellow discoloration of the hair was a frequent observation in F<sub>0</sub> adult males (9%) and females (27%), and in F<sub>1a</sub> progeny (87%/males, 82%/females) at the high dose. It was also present in F<sub>2</sub>→F<sub>3a</sub> progeny at the high dose (40/119= 34% in males; 31/93=33% in females).

A similar effect to that seen in the behavioral data on the pulmonary system was noted at the mid and high doses in both the adult males and females of the F<sub>1</sub> generation, i.e., pulmonary subpleural grey foci: 12% and 20%/cont.; 24% and 27%/mid; 28% and 53%/high, respectively (see Table 7). In the F<sub>2</sub> adults the males also exhibited a similar gross observation at the high dose (13%/cont., 53%/high) while the large number (up to 50%) of females reported as dead prevented any determination of a dose-related effect. Microscopic data gave no indication of any internal lung lesions.

Table 7: Selected Gross Necropsy Data

Group's sex(#)	0 mg/kg/day		1 mg/kg/day		3 mg/kg/day		10 mg/kg/day	
	males	females	males	females	males	females	males	females
F <sub>1</sub> adults	(25)*	(15)	(25)*	(15)	(25)*	(15)	(25)*†	(17*)
pulmonary sub-pleural grey foci	3(12%)	3(20%)	3(12%)	4(27%)	6(24%)	4(27%)	7(28%)	9(53%)
(* 1 rat found dead, † 1 rat sacrificed due to mis-sexing)								
F <sub>2</sub> adults (#)	males	females	males	females	males	females	males	females
	(15)*	(5) <sup>b</sup>	(15*)	(4) <sup>b</sup>	(5)*	(5) <sup>c</sup>	(15)*†	(5*) <sup>a</sup>
pulmonary congestion	2(13%)	5(100%)	1(7%)	4(100%)	3(20%)	3(60%)	8(53%)	2(40%)
pulmonary sub-pleural grey foci	1(7%)	—	—	—	1(7%)	—	5(33%)	2(40%)
(*1 rat dead; *†two rats dead; <sup>a</sup> 3 rats dead; <sup>b</sup> 4 rats dead; <sup>c</sup> 5 rats dead)								

#### DISCUSSION

The reproductive (pre- and post-natal) and teratogenic effects of continuous feeding (diet) of dinoseb to rats at 0, 1, 3, 10 mg/kg/day dosages have been studied in a three generation (two matings per generation) study.

There is a consistent, compound-related decrease in body weight gain at the high dose in both adult males and females in the pre-mating period in all three generations, which continues in the treated males and females during mating, post-mating, etc. at the high dose concentration. Although the mean weight gains fluctuate considerably, the males continue to exhibit a lower weight at the high dose than the controls during the period from mating to the study's completion. There continues to be a consistent but slight decrease in female weights during the gestation period in the a and b matings in all three generations at the high dose.

Examination of the mean fetal indices indicates that fetal weights were affected by dinoseb administration, but not consistently, throughout the generations studied. Decreased weight gains appear to occur in three of the littering groups excluding F<sub>1a</sub>, F<sub>2b</sub>, F<sub>3b</sub>. F<sub>0</sub>→F<sub>1b</sub> pup weights were diminished (combined sexes) at day 21 at all dose levels compared to controls and the per cent weight increases were statistically significantly lower at all dose levels (p<0.05). This is reflected by the lower pup weight gains seen in the individual sexes at day 21 and indicates an effect of dinoseb on the pups during lactation since the pup weights at birth were similar. Based on the findings for pup weights (decreased), a reproductive LEI of 1 mg/kg/day is determined.

Although not statistically significant, similar effects to those in the F<sub>0</sub>→F<sub>1b</sub> littering groups were noted in the F<sub>1</sub>→F<sub>2a</sub> pups weights at day 2, again suggesting a compound-related effect during the lactation period. The toxicity of dinoseb in the F<sub>2</sub>→F<sub>3a</sub> littering group is different in that a statistically significant decrease in pup weight(g) at day 1 at all dose levels is observed as com-

pared to control values. This depressed effect on weight gain remains at day 4 and at day 21.

Examination of the Caesarian data indicates variable effects for maternal toxicity and fetotoxicity in treated animals as compared to controls.

The maternal data indicate some compound-related effects, primarily in the  $F_0$  data. A statistically significant ( $p < 0.05$ ) decrease in the mean number of functional corpora lutea at the high dose was noted, resulting in a lower (nss) number of implants per pregnant dam. There were a number of significant changes in the pre-implantation losses in all generations but their meaning is uncertain in light of the large variation in the controls (ranged from 2.8 to 17.2%). There was a higher percentage of pre-implantation losses in all dose groups ( $F_0$ ) as compared to the controls, with the mid-dose being statistically significant. In the  $F_1$  data there was a large increase at the low dose in percentage pre-implantation loss while there appeared to be a diminution (nss) in the % pre-implantation loss in all dose groups of the  $F_2$  generation as compared to the controls. Since the control value was on the upper end of the control range for pre-implantation losses the diminution may not be a real effect.

Possible compound- and/or dose-related toxicity was observed for all offspring of the  $F_0$  and  $F_2$  generations. In the  $F_{1b}$  pups there was a suggestion of a dose-related increase in the overall skeletal defects (% "minor" fetal defects) as compared with the control which was statistically significant at the high dose, while the % "variants" were increased (nss) only in the low dose group. For the  $F_{3b}$  pups there was an apparent compound-related increase (nss) in the total number of "minor" skeletal defects, primarily from an increase (treatment-related) in sternebral and rib defects. Variants (forelimb or hindlimb phalanges incompletely/not ossified) were consistently increased in a compound-related fashion (statistically significant at mid- and high-dose levels).

Behavioral data, while suggestive of post-natal toxicity of a compound- or dose-related nature are difficult to interpret. No significant post-natal toxicity is ascribed to dinoseb administration at the doses studied in this assay in light of the small number of animals studied per group, the finding of a small weight change in only one group of rats ( $F_{1b}$  males), and the lack of consistent, statistically significant effects.

While the findings of fetotoxicity are of qualitative importance, there are a number of reasons why a NOEL cannot be established in this study. First, the small number of dams utilized (9 to 10) precludes the determination of fetotoxicity with any statistical confidence. Furthermore, the investigators did not present litter incidence for fetal defects. Finally, the pre-implantation loss is quite variable in the controls, making interpretation of the findings uncertain.

With regards to the methodology for the study, a major deficiency in the study was the significant variability of the estimated dosages fed to the animals during the study as well as uncertainty regarding the analysis of the content of the fortified diet and the concentration of compound actually present in the diet. In addition, the report indicated the loss of food records for weeks 9 and 14-65 for both males and females which precludes an accurate estimate of the administered dose.

This study is classified Core Supplementary data.

## B. 2 Generation Reproductive Study

STUDY TYPE: Two generation reproductive study in the rat

CHEMICAL: Dinoseb, 2-sec butyl 4,6-dinitrophenol

TEST MATERIAL: Technical grade dinoseb; brown crystalline solid (batch # M1 2000-25, AGR number 133942) of 98.0% purity; blended with the basic powdered diet in a Gardner 3C double cone blender.

### STUDY IDENTIFICATION:

a. Title: 2-Sec-butyl-4,6-dinitrophenol (dinoseb) additional 2 generation phase of a 3 generation reproductive performance study in the rat (dietary)

b. Laboratory: Hazleton Laboratories Europe Ltd.,  
Otley Road,  
Harrogate, HG3 1PY,  
England

c. Study Number: 2350-50/58

d. Study Date: April 1981

e. Study Director: L.F.H. Irvine, B.Sc.  
Department of Small Animal Toxicology

f. Caswell # 392DD; Accession # 259499-259506; EPA # 54299-Q (2)

### CONCLUSIONS:

In light of: 1) the low viability index for pups in the F<sub>4</sub>→F<sub>5a</sub> controls (which does not allow a useful comparison of the fetal control data to the treated groups), 2) the inconsistency between the weight changes in the present study (significant weight increases) and the previously reviewed study (significant decreases in three of the six littering groups), and 3) the consistent decrease observed in gonadal weights and organ-to-body weight ratios at all dose levels, it is concluded that a NOEL for reproductive toxicity in the pups can not be established. In addition, the study has failed to establish a systemic NOEL for the depressed weight gains observed in the adults (males or females) and the LEL for systemic toxicity is 1 mg/kg/day (LDT), based on this effect.

An important deficiency in the methods is the lack of stability data on the stock dinoseb from which the animals were dosed in the feed.

This study is designated as Core Supplementary data.

METHODS:

A photocopy of the methods section has been appended. The following comments are noted:

1. In contrast to the first reproduction study (3-generation, two matings/generation) submitted (see page 1 of this review), this present study only has a single mating per generation (F<sub>4a</sub>, F<sub>5a</sub> pups).
2. The investigators noted that animals (adults; end of F<sub>3</sub> generation phase; p.10, volume XI) were moved from one animal room(1) to another room (25).
3. Only the following complete or gross necropsies were performed:

complete necropsy (gross necropsy/microscopic)

10 male and 10 female F<sub>3</sub> and F<sub>4</sub> adults

5 male and 5 female F<sub>4a</sub> pups

gross necropsy only

surplus F<sub>3</sub> and F<sub>4</sub> adults

surplus F<sub>4</sub> pups

all F<sub>5a</sub> pups

The 1978 Guidelines stated that 10 male and female F<sub>1</sub> adults were to be subjected to a complete gross necropsy and histopathology examination. The 1982 EPA Guidelines request full histopathology on the vagina, uterus, ovaries, testes, epididymus, seminal vesicles, prostate and target organ(s) for all high dose and control P<sub>1</sub> and F<sub>1</sub> (equivalent to F<sub>3</sub> and F<sub>4</sub> of this study) animals selected for mating.

4. As noted in the first reproduction study reviewed, the stability and homogeneity of the test substance are of particular importance in a long-term test:

a. It is unclear as to whether the stability of technical dinoseb per se was determined; this is critical since all the dietary mix was prepared from a single batch of test material shipped from the manufacturer, Dow Chemical Pacific Ltd (p. 11, volume XI). Edgerton and Moseman (J.Agric.Chem.,26(2):425,1975) observed that their 2-sec-butyl-4,6-dinitrophenol (DNBP) analytical standards (liquid form) significantly degraded(27% loss after 72 hrs) when stored in clear glass bottles. Were the internal standards of DNOC (4,6-dinitro-o-cresol) and DNBP used in the calibration curves and subsequent dietary stability tests (DNOC only) adequately controlled for chemical degradation over the life of the study?

b. Stability studies were performed for dinoseb in the feed (pages 107-109, Vol. XI) stored at room temperature (+ 22°C) or frozen (-20°C). Mean values were presented but no indication of the variability of the samples analyzed were presented, e.g., standard deviations.

c. The analytical results from samples of dinoseb incorporated into the diet (p. 114, volume XI) indicate a considerable variation in the concentration of dinoseb recovered:

<u>week #/generation</u>	<u>% spiking level</u>
3/F <sub>3</sub>	87.5 - 117.0
1/F <sub>4</sub>	41.0 - 112.9
22/F <sub>4</sub>	59.5 - 138.4

This is apparently the result of poor mixing of the test diet and not poor analytical recovery. Edgerton and Moseman (1973) noted that they obtained poor recovery in their DMBP fortified diet after 2 days (apparent loss of 28%) unless the compound was extracted using acid hydrolysis; however, acid hydrolysis was employed in this study (based on the analytical description discussed in the mouse oncogenicity study; Volume I, p.135).

d. The calculation for dinoseb intake (pages 3, 4; vol. XII, XIII) indicates that there was considerable variability in the doses administered based on the weekly diet intake and that the nominal dosages are only a rough estimate of the test article the rats received. The following is an average of the weekly records reported:

	<u>Calculated dosage ( mean average in mg/kg/day)</u>		
	<u>1 mg/kg/day</u>	<u>3 mg/kg/day</u>	<u>10 mg/kg/day</u>
<u>F<sub>3</sub>: males</u>			
<u>period/week</u>			
pre-mating (1-14)	1.093(.85-1.40)	3.19(2.3-4.74)	10.6(8.5-13.56)
mating (15, 16)	0.72(.64,.75)	2.035(1.83,2.24)	6.11(5.43,6.78)
post-mating (17-23)	0.967(.78-1.14)	2.86(2.34-3.3)	9.86(8.22-12.47)
<u>F<sub>3</sub>: females</u>			
<u>period/week</u>			
pre-mating (1-14)	1.085(.83-1.48)	3.26(2.61-4.4)	10.77(8.31-15.76)
mating (15, 16)	1.2(1.09,1.3)	3.54(3.24,3.83)	11.4(10.32,12.47)
gestation/lactation* (17-23)	1.49(1.07-2.0)	4.634(3.73-5.14)	15.69(11.88-18.11)
<u>F<sub>4</sub>: males</u>			
<u>period/week</u>			
pre-mating (1-14)	1.04(.85-1.3)	3.11(2.56-4.41)	10.59(7.98-13.57)
mating (15, 16)	0.63(.53,.73)	1.84(1.74,1.94)	6.37(4.98,7.76)
post-mating (17-22)	0.96(.86-1.13)	2.82(2.2-3.56)	9.75(8.31-10.77)
<u>F<sub>4</sub>: females</u>			
<u>period/week</u>			
pre-mating (1-14)	1.11(.82-1.52)	3.24(2.47-4.6)	10.81(3.17-15.41)
mating (15, 16)	1.045(.9,1.19)	3.18(3.06,3.3)	10.56(8.42,12.69)
gestation/lactation* (17-22)	1.41(.77-1.78)	4.39(1.99-5.48)	13.74(8.43-17.00)

\* stated as elevated due to wastage and offspring feeding on diet

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5. The vagina, uterus, and seminal vesicles were not examined as requested in both the 1978 Proposed and 1982 Final Guidelines for Toxicology Testing.

## RESULTS

### 1. Group Mean Parental Body Weight Gain: Table 1

A lower weight gain is observed in the F<sub>3</sub> males in the pre-mating period at all dose levels as compared to the controls, which is statistically significant at the low and high doses (399=control; 357=low,  $p<0.01$ ; 376=mid; 367=high,  $p<0.05$ ). The F<sub>3</sub> adult females had a statistically significant lower weight gain than the controls at only the high dose (193=control; 178=high,  $p<0.05$ ). The lower weight gains continued in both sexes during the post-mating period. The male weights were statistically significant lower, again at the low and high doses (469=control, 414=low,  $p<0.01$ ; 455=mid, 421=high,  $p<0.05$ ). In the females, body weights were statistically significantly different from controls at all doses during gestation (124=control; 113=low,  $p<0.01$ ; 109=mid,  $p<0.05$ ; 108=high,  $p<0.05$ ).

A similar effect to that noted in the F<sub>3</sub> males, but which was not a statistically significant lower weight gain as compared to the controls, was reported in the F<sub>4</sub> males during the pre-mating period at the low and high dose levels (342=control, 332=low, 358=mid, 320=high). During gestation a lower weight gain (statistically significant) was noted in the F<sub>4</sub> females at the high dose (108=control, 88=high,  $p<0.01$ ), while in the F<sub>4</sub> males a similar weight gain pattern to the pre-mating period was observed in the post-mating period, that is, a lower weight gain at the low and high doses than in the controls (423=control, 405=low, 437=mid, 384=high,  $p<0.05$ ).

### 2. Group Mean Reproductive Indices: Table 2

Group mean reproductive indices are presented in Table 2 below. No significant effects were noted upon examination of the adult data for male and female fertility, or the gestation index--a measure of the number of pregnant females with live pups for either F<sub>3</sub> or F<sub>4</sub> generations at any dose level. Further, there was no indication of any real compound-related decrease in fetal viability in any generation as measured by the live birth index (measure of pup viability at birth), viability index (pup viability at day 4), or lactation index (pup viability at weaning). However, there was a consistent decrease in the viability indices at all doses, including the controls (30%), in the F<sub>4</sub>→F<sub>5a</sub> litter. In comparison, the viability index for the F<sub>3</sub>→F<sub>4a</sub> female controls was 95%, a value comparable to the viability indices for controls in the previously reviewed 3-generation reproductive study (ranging from 92 to 100%). Therefore, the F<sub>4</sub>→F<sub>5a</sub> control value appears too low to allow a useful comparison to the dinoseb-treated groups.

Table 1: Group mean parental body weight gains(g)

Period	0 mg/kg/day	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
<u>[F<sub>3</sub>]</u>				
Pre-mating(1-15wks)				
Males	399	357**	376	367*
Females	193	194	202	178*
<u>(F<sub>4a</sub>)</u>				
Gestation(0-21 days)				
Females	124	113***†	109*††	108*††
Lactation(1-21 days)				
Females	-13	+5	-4	0
Post-mating(1-24wks)				
Males	469	414****	455	421***
Postlactation (1-24wks)				
Females	199	220	216	196
<u>Period</u>				
<u>[F<sub>4</sub>]</u>				
Pre-mating(1-15wks)				
Males	342	332	358	320
Females	162	170	180	167
<u>(F<sub>5a</sub>)</u>				
Gestation(0-21 days)				
Females	108	118	117	88**
Lactation(1-21 days)				
Females	+9	+20	+24	+20
Post-mating(1-23wks)				
Males	423	405	437	384*
Postlactation (1-23wks)				
Females	203	222	222	205

F<sub>3</sub>: \*weight gain weeks 1-15 significantly lower than controls (p<0.05; t test);

\*\*weight gain weeks 1-15 significantly lower than controls (p<0.01; t test)

\*\*\*weight gain during gestation significantly lower than controls (p<0.05; t

test) or (p<0.01; t test), resp.; \*\*\*weight gain during gestation significantly lower than controls (p<0.01; t test)

F<sub>4</sub>: \*weight gain during weeks 1-23 significantly lower than controls (p<0.05; t test); \*\*weights during gestation significantly lower than controls (p<0.05; t test)

Table 2: Group Mean Reproductive Indices

Parameter	0 mg/kg/day	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
<u>F<sub>3</sub>→F<sub>4a</sub></u>				
# animals mated	24	25	24	23
# animals not mated	1	0	1	2
# pregnancies	24	25	21	23
mating index	92.3	100.0	100.0	88.5
fecundity index	100.0	100.0	87.5	100.0
male fertility(F <sub>3</sub> )	100.0	100.0	100.0	100.0
female fertility(F <sub>3</sub> ) <sup>†</sup>	100.0	100.0	87.5	100.0
gestation index	100.0	96.0	100.0	95.7
live birth index	100.0	100.0	99.6	99.6
viability index	94.9	95.8	85.3	96.7
lactation index	96.8	96.4	97.0	98.3
<u>F<sub>4</sub>→F<sub>5a</sub></u>				
# animals mated	23	25	24	24
# animals not mated	2	0	1	1
# pregnancies	22	25	24	21
mating index	100.0	92.6	100.0	92.0
fecundity index	95.6	100.0	100.0	87.5
male fertility(F <sub>4</sub> )	95.4	100.0	100.0	95.2
female fertility(F <sub>4</sub> )	88.0	100.0	96.0	84.0
gestation index	95.5	100.0	95.8	95.2
live birth index	100.0	100.0	100.0	100.0
viability index	69.7	71.8	65.0	74.6
lactation index	93.7	93.5	85.6	95.1

<sup>†</sup> this parameter was incorrectly presented in the report as the number of pregnancies divided by the number of females exposed to males

### 3. Group Mean Fetal Indices (littering group): Table 3

A statistically significant higher ratio (larger number) of females in the high dose group is seen in the F<sub>4a</sub> fetuses as compared to the controls (1:0.86=cont., 1:1.35, p<0.05) which is not statistically significant (but is increased) in the F<sub>5a</sub> female fetuses also (1:0.99=cont., 1:1.11=high). The meaning of this effect is uncertain and the reviewer is reluctant to attribute any biological significance to it.

The data presented in Table 3 for body weight changes are difficult to interpret. In contrast to a diminution in weight observed in fetuses exposed to dioxin in the previously reviewed reproductive study (see review on 3-generation reproductive study), a statistically significant increase in pup weight(g) at day 21 was observed in the F<sub>4a</sub> pups at the low dose as compared to controls (29.3=cont., 35.0=low, p<0.01). An increase was also seen in the pup weight of the F<sub>5a</sub> progeny at day 1 (statistically significant at low dose: 5.6=control; 6.0=low, p<0.01; 5.7=mid; 6.1=high), at day 4 (statistically significant at high dose: 7.0=control; 7.5=low; 7.0=mid; 8.1, p<0.01) and at day 21 (statistically significant at low dose: 29.3=control; 33.5=low, p<0.01; 29.5=mid; 32.2=high). Apparently the weight increases are not related to significant decreases in the general litter size of the present study(which might skew the body weight changes), as

evidenced by similar control mean litter sizes in the initial three generation reproductive study [which ranged from 13.8 to 10.3 fetuses/dam as compared to this study where there were 12.2 ( $F_{4a}$ ) and 10.9 ( $F_{5a}$ ) fetuses/dam in the controls]. There was a statistically significant reduction in the  $F_{4a}$  litter size of the high dose group and a suggestion of a similar reduction in the high dose group of  $F_{5a}$ .

#### 4. Reproductive Organ(Gonads) Weights/Organ-to-Body Weight Ratios: Table 4

Reproductive organ (gonads) weights/organ-to-body weight ratios are presented in Table 4 for the  $F_3$  and  $F_4$  adults and the  $F_{4a}$  pups ( $F_{5a}$  data not reported).

There appears to be a small, treatment-related decrease in ovary weights at the mid- and high-dose levels  $F_3$  females. A statistically significant decrease in the ovaries (total) of the  $F_3$  adult females at the mid-dose was observed for both absolute organ weights and organ-to-body weight ratios (.102/.039=control; .082/.03=mid,  $p < 0.05$  for both parameters). The high dose weights were lower than the controls also (0.092/.034=high). No significant changes were observed in the  $F_3$  adult male or the  $F_4$  adult male or female organ weights or organ-to-body weight ratios.

In the  $F_{4a}$  progeny, there was a consistent decrease (not statistically significant) in the left, right and total gonad weights and organ-to-body weight ratios at all dose levels in the male pups but not the females, e.g., the weight totals (absolute/relative): .726/1.022=control, .696/.872=low, .668/.943=mid, .549/.845=high).

#### 5. Gross Necropsy and Microscopy

No unusual findings were observed on gross necropsy of the  $F_3$  adult males and females (appendices 11, 12, volume XII). The histopathological data for the adult animals did not show any unusual effects except for a slight increase in lymphocytic perivascularitis in males and females (4/10, 4/10:cont.; 4/10, 6/10:low; 6/10, 8/10: mid; 7/10, 6/10: high, respectively). There is also a suggestion of an effect in the submaxillary gland of males for increased diffuse sialoadenitis (1/10:cont. 2/10:low, 6/10:mid, 6/10:high) and diffuse periglandular edema (1/10:cont., 2/10: low, 5/10:mid, 6/10:high). Lymphadenitis of the mandibular lymph nodes in males also appeared to be increased (0/10:cont., 1/10:low, 3/10:mid, 6/10:high). No unusual findings were noted in the  $F_{4a}$  rat pups selected for histopathological examination.

No unusual findings were reported on gross necropsy of the  $F_4$  adult males and females or the  $F_{5a}$  pups( appendices 9-11, volume XIII). The histopathological data for the adult animals showed an increase in the staining of the skin (yellow) at the mid and high dose levels in both the males and females (1/10, 3/10:mid; 10/10, 10/10:high, respectively). This relates to the color of the compound.

Table 3: Mean Fetal Indices (Littering group)

<u>F<sub>3</sub>→F<sub>4a</sub></u>	0 mg/kg/day	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
ratio males:females	1:0.86	1:0.80	1:0.92	1:1.35**
mean litter size <sup>a</sup>	12.2(1.89)	11.0(2.29)	11.1(2.57)	11.0(2.06)***
pup wt. day 1(g)†	6.0	5.9	5.8	5.9
pup wt. day 4(g)/	8.7	8.7	8.7	8.3
% wt. increase	45.0	47.5	50.0	40.7
pup wt. day 21(g)/	29.3	35.0*	30.5	29.6
% wt. increase over pre-weaning period	388.3	493.2	425.9	401.7
male pup wt. day 21(g)	29.6	35.4	30.8	30.0
female pup wt. day 21(g)	28.8	34.8	30.2	29.1

F<sub>4</sub>→F<sub>5a</sub>

ratio males:females	1:0.99	1:0.86	1:1.00	1:1.11
mean litter size <sup>a</sup>	10.9(2.26)	11.1(3.24)	12.0(2.67)	9.7(3.34)
pup wt. day 1(g)†	5.6	6.0*	5.7	6.1
pup wt. day 4(g)/	7.0	7.5	7.0	8.1*
% wt. increase	25.0	25.0	22.8	32.8
pup wt. day 21(g)/	29.3	33.6*	29.5	32.2
% wt. increase over pre-weaning period	423.2	460.0	417.5	427.9
male pup wt. day 21(g)	29.8	33.5	30.1	32.5
female pup wt. day 21(g)	28.8	33.2	28.8	31.5

† values include data from those animals with live pups of a particular sex on each day; <sup>a</sup> number of pups per dam (S.D.), for comparison purposes the mean litter sizes in the controls of the previously reviewed 3-generation reproductive study were: F<sub>0</sub>→F<sub>1a</sub>= 13.2(2.28), F<sub>0</sub>→F<sub>1b</sub>= 12.7(2.29); F<sub>1</sub>→F<sub>2a</sub>= 13.1(2.12), F<sub>1</sub>→F<sub>2b</sub>= 13.8 (1.97) ; F<sub>2</sub>→F<sub>3a</sub>= 10.3(2.48), F<sub>2</sub>→F<sub>3b</sub>= 10.5(3.53)

F<sub>3</sub>→F<sub>4a</sub>: \*significantly higher than in controls(p<0.01: Wilcoxon's test); \*\*sex ratio significantly different from controls(p<0.05: Wilcoxon's test) \*\*\* significantly lower than in the control group (p<0.05: Wilcoxon's test)  
F<sub>4</sub>→F<sub>5a</sub>: \*significantly higher than in controls(p<0.05: Wilcoxon's test)

Table 4: Organ weights/organ-to-body weight ratios: reproductive organs

<u>F<sub>3</sub>→F<sub>4</sub></u>		<u>Gonads(mean(g),(S.D.)/organ-to-b.wt. ratio[%],(S.D.))</u>		
<u>Adults</u>		<u>Left</u>	<u>Right</u>	<u>Total</u>
1M		1.7(.186)/.350(.037)	1.7(.182)/.347(.038)	3.4(.367)/.697(.074)
1F		.050(.014)/.019(.005)	.051(.013)/.020(.005)	.102(.026)/.039(.009)
2M		1.8(.148)/.380(.050)	1.8(.107)/.381(.053)	3.5(.249)/.761(.102)
2F		.052(.013)/.019(.005)	.045(.010)/.017(.004)	.098(.019)/.036(.007)
3M		1.7(.142)/.328(.030)	1.7(.159)/.329(.030)	3.4(.295)/.657(.059)
3F		.039(.007)/.014(.003)	.043(.013)/.016(.005)	.082*(.016)/.03*(.006)
4M		1.6(.315)/.342(.065)	1.6(.399)/.334(.091)	3.2(.412)/.676(.097)
4F		.048(.012)/.018(.005)	.045(.009)/.016(.004)	.092(.019)/.034(.007)
<u>F<sub>4a</sub> progeny</u>				
1M		.360(.062)/.507(.079)	.365(.058)/.514(.074)	.726(.120)/1.022(.153)
1F		.012(.001)/.017(.002)	.011(.001)/.015(.001)	.023(.002)/.032(.003)
2M		.346(.084)/.434(.079)	.350(.086)/.438(.078)	.696(.170)/.872(.158)
2F		.009(.002)/.010(.002)	.008(.002)/.010(.002)	.017(.004)/.020(.002)
3M		.334(.060)/.471(.082)	.335(.062)/.472(.084)	.668(.121)/.943(.167)
3F		.011(.002)/.015(.003)	.010(.002)/.014(.004)	.022(.003)/.029(.007)
4M		.275(.134)/.424(.139)	.274(.133)/.421(.142)	.549(.267)/.845(.280)
4F		.011(.007)/.016(.011)	.008(.003)/.011(.005)	.019(.010)/.028(.016)

\* significantly different from the control (p&lt;0.05: t test)

(table continued on next page)

Table 4: Organ weights/organ-to-body weight ratios: reproductive organs  
( continued)

<u>F<sub>4</sub>→F<sub>5</sub></u>	<u>Gonads(mean(g), S.D./organ-to-b.wt. ratio(%), S.D.)</u>		
	<u>Left</u>	<u>Right</u>	<u>Total</u>
Adults			
1M	1.55(.208)/.309(.047)	1.58(.175)/.316(.036)	3.13(.354)/.625(.079)
1F	.038(.013)/.013(.004)	.042(.017)/.015(.006)	.079(.028)/.028(.009)
2M	1.7(.210)/.339(.051)	1.65(.093)/.33(.038)	3.35(.285)/.669(.084)
2F	.040(.009)/.014(.004)	.039(.008)/.014(.003)	.079(.015)/.027(.006)
3M	1.5(.137)/.293(.027)	1.52(.141)/.296(.026)	3.02(.274)/.589(.052)
3F	.039(.012)/.013(.004)	.041(.018)/.013(.006)	.080(.028)/.026(.009)
4M	1.48(.172)/.306(.021)	1.49(.159)/.309(.02)	2.95(.329)/.615(.04)
4F	.045(.007)/.016(.003)	.044(.008)/.015(.003)	.089(.013)/.031(.005)

#### DISCUSSION

The reproductive effects of continuous feeding (diet) of dinoseb to rats at 0, 1, 3, 10 mg/kg/day dosages have been studied in a two generation/single littering per generation study where the first parental generation adults have been derived from the F<sub>2</sub>→F<sub>3</sub> offspring of a previously initiated three-generation reproductive study, i.e., the "F<sub>0</sub>" and "F<sub>1</sub>" generations are actually the F<sub>3</sub> and F<sub>4</sub> generations (see methods section).

Dinoseb administration produced treatment- and/or dose-related reductions in maternal or paternal weights including: 1) a lower weight gain in the F<sub>3</sub> males in the pre-mating period at all dose levels as compared to the controls which is statistically significant at the low and high doses; the adult females had a statistically significant lower weight gain than the controls at only the high dose, 2) lower weight gains in the F<sub>3</sub> males during the post-mating period being statistically significant again at the low and high doses and in the females being statistically significantly different from controls at all doses during gestation, 3) a similar effect to that noted in the F<sub>3</sub> males but which was not a statistically significant lower weight gain as compared to the controls was reported in the F<sub>4</sub> males during the pre-mating period at the low and high dose levels, and 4) during gestation a lower weight gain (statistically significant) was noted in the females at the high dose while in the males a similar weight gain pattern to the pre-mating period was observed in the post-mating period, that is, a lower weight gain at the low and high doses than in the controls. There was also an apparent treatment-related reduction in total ovary weights (F<sub>3</sub> females) for absolute and organ-to-body weights ratios at the mid- and high-dose levels which was statistically significant at the mid dose.

It is difficult to interpret the effect of dinoseb administration observed on the offspring. The  $F_4 \rightarrow F_{5a}$  control value appears too low to allow a useful comparison to the treated groups which are all decreased to a similar degree also. In regard to fetal weight changes, in contrast to a diminution in weight observed in fetuses exposed to dinoseb as previously noted in the review on a 3-generation reproductive study, a statistically significant increase in pup weight at day 21 was observed in the  $F_{4a}$  pups at the low dose as compared to controls. An increase was also seen in the pup weight of the  $F_{5a}$  progeny at day 1 (statistically significant at low dose), at day 4 (statistically significant at high dose) and at day 21 (statistically significant at low dose). This is not related to lower litter sizes which might skew the weight changes. In the  $F_{4a}$  progeny, there was a consistent decrease (not statistically significant) in the left, right and total gonadal weights and organ-to-body weight ratios at all dose levels in the male pups but not the females.

In light of: 1) the low viability index for pups in the  $F_4 \rightarrow F_{5a}$  controls (which does allow a useful comparison of the fetal control data to the treated groups), 2) the inconsistency between the weight changes in the present study (significant weight increases) and the previously reviewed study (significant decreases in three of the six littering groups), and 3) the consistent decrease observed in gonadal weights and organ-to-body weight ratios at all dose levels, it is concluded that a NOEL for reproductive toxicity in the pups can not be established. In addition, the study has failed to establish a systemic NOEL for the weight changes observed in the adults (males or females) and the LEL for systemic toxicity is 1 mg/kg/day (LDT).